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EXPERIMENTAL INVESTIGATIONS ON THE ORIGIN AND DETERMINATION OF THE GERM CELLS, AND ON THE DEVELOPMENT OF THE LATERAL PLATES AND GERM RIDGES IN URODELES

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GENERAL INTRODUCTION.

The problem of the origin of the germ cells in the Vertebrates is a very old one. In the 19th century almost all the investigators published extensive descriptions of the normal development of eggs and larvae from which many interesting facts became known. Several important questions, however, remained unsolved, especially the earliest development could not be elucidated by this descriptive method, as the characteristics of the various cells are still unvisible at those stages. Moreover, other questions, for instance concerning the causes of the succession of the various developmental phases and the possibility of a determination of the germ cells, could not be explained; they can only be approached experimentally.

At the beginning of the present century the majority of investigators still made use of a more and more scrupulous microscopical analysis of the early normal development; without, however, obtaining conformable results. But a few authors used the experimental method. They have already recorded important data. As the solution of many other problems was still obscure, I decided to solve the problem of the origin of the germ cells experimentally. I will give the results of these investigations in the following pages.

In the course of these experiments on the origin of the germ cells, many interesting facts have been established in respect to the determination of the germ ridges and the development of the lateral plate. I, therefore, extended the field of my investigations to these subjects.

As it was evident that the germ cells originate from the mesoderm of the presumptive lateral plates, the development of the

latter is of so great importance that I will treat its determination and development in a separate part. The same holds good for the development of the germ ridges.

In order to proceed systematically, I will first deal with the determination of the lateral plates, thereafter with the origin, development and determination of the germ cells and, finally, with the development and determination of the germ ridges. The second part is divided into three chapters, *viz.*, the origin of the germ cells, their determination, and their migration. Cf. the contents of this paper.

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MATERIAL AND METHODS.

As the most important investigations on the origin of the germ cells (BOUNOURE (1924-1937), MONROY (1939) etc.) have been made in the Anures, it would have been obvious to use animals of the same group in my experiments. The Anurian eggs, however, do not lend themselves very well to experimental investigation, as the egg membranes are very difficult to remove, especially in the early stages of development.¹⁾ Urodelian eggs, however, may

¹⁾ A method was worked out by HLUCHOVSKI (1927) to remove the membranes of the Anurian eggs more easily after ultra-violet irradiation. In how

easily be delivered from their capsules by mechanical methods; after some practice, this may be done without any injury to the eggs. I therefore used eggs of the native species *Triturus taeniatus* (Schneid.), *Tr. alpestris* (Laur.) and *Tr. cristatus* (Laur.)¹ and of the exotic Axolotl, *Ambystoma mexicanum* (Cope).

The three native species were freshly caught in spring, and kept in small aquaria. A few branches of the aquatic plant *Elodea canadensis* were used as a substrate, on to which the newts readily attached their eggs.

In captivity *Triturus taeniatus* and *Tr. alpestris* deposited their eggs as a rule during several weeks, whereas *Tr. cristatus* usually gave only one set of eggs, and then stopped laying. It was, therefore, necessary to replace the *Tr. cristatus* animals by new ones from time to time. This rendered it difficult to get a sufficiently large number of eggs from one animal, which is desirable for quantitative experiments (cf. p. 73).

Triturus cristatus, kept in captivity, laid eggs which often showed an abnormal development. Usually some of the eggs had not been fertilized, of others development stopped at an early gastrula stage. The embryos of still others, after reaching the tail bud stage, showed oedema vesicles and died in a few days. Thus, many experimental animals were eliminated during the rearing of the larvae. Fortunately, however, hardly any eggs of *Triturus taeniatus* and *Tr. alpestris* showed these irregularities. *Triturus cristatus* eggs, therefore, were used only in the transplantation experiments between different species of *Triturus* (heteroplastic). The other experiments were made on the eggs of *Triturus taeniatus*, *Tr. alpestris* or *Ambystoma mexicanum*.

The Axolotls were kept in rather large aquaria. In order to obtain eggs, ♂ and ♀ were kept apart for a week at a temperature of 20°–23° C, and then brought together in a large aquarium at a temperature of 10° C. The decline in the temperature gives an impulse to the deposition of the spermatophores and eggs. *Ambystoma mexicanum* yields a large number of eggs at a time. The eggs were kept in the ice-box at 6–7° C. At this temperature development proceeds very slowly, with the result that the eggs of one batch may be used for a fairly long time. The eggs of the *Triturus* species can be kept in the ice-box for a few days only, as the early developmental stages especially do not develop normally below 10° C.

In order to remove the capsules from the eggs, I sometimes used SPEMANN's method (1923) in which a part of the shell is cut off with iridectomy scissors. In most cases, however, I prepared the eggs with two pairs of sharp watch-maker's forceps. By

far this treatment is injurious to the eggs is, however, unknown, and will be very difficult to determine. From the investigations of BOUNOURE we know that ultra-violet rays have a marked influence on the development and on the number of the germ cells. Thus, a treatment with ultra-violet light might affect the normal development in such a way that we should draw entirely wrong conclusions from our experiments.

¹) The commonly used names are *Triton taeniatus*, *Tr. alpestris* and *Tr. cristatus*, respectively.

this method the removal of the membranes can be carried out with great precision, and in this way it is possible to deliver the eggs without any injury. As the yolk membrane and the eggs are very delicate in *Triturus cristatus*, only the second method must be used here. The eggs of *Ambystoma mexicanum* were also prepared by means of the second method, since their egg membranes are too thick and gelatinous to make use of the first.

The operation technique, as worked out by SPEMANN (1923), has been employed. Almost all *Triturus* embryos were fixed at stage 42/43 (GLAESNER). At this stage, the yolk material has been entirely consumed, but the larvae can still develop normally without food. The *Ambystoma* larvae were fixed at the corresponding stage (± 25 days after the operation). Then their intestine is entirely empty and of small diameter, which facilitates the counting of the germ cells in the germ ridges.

ZENKER solution was used as fixative. The larvae were embedded in paraffin in the usual way via alcohol and benzol. Their preparations were sectioned at $8\ \mu$, and stained in DELAFIELD's hematoxylin and eosin. The embryos with heteroplastic grafts were stained in DELAFIELD's hematoxylin only; the brown pigment granules, the difference in the amount of which is one of the characteristics of the species used, are better visible without red plasma staining. Cf. p. 120-122.

PART I - THE DETERMINATION OF THE LATERAL PLATES IN THE URODELES.

A) INTRODUCTION.

In the course of my investigations on the origin of the germ cells in the Urodeles, an experiment was made in which the whole entoderm was removed at an early neurula stage. These embryos, which grew very well and in which differentiation proceeded normally, showed very interesting alterations in the development of the lateral plates. In the normal development the lateral plate is divided by the coelomic cavity into two layers, an outer and an inner one, which develop into a somatopleure and a splanchnopleure, respectively. On the contrary, in the experimental animals, both layers had formed somatopleures with all their characteristics, such as forelimbs, muscle layers, chromatophores, spinal nerves, etc.

Although BALINSKY (1939a) performed the above operation in the same way, he has not recorded anything concerning the development of supernumerary forelimbs. Neither has he mentioned any other fact to indicate that he had observed the inner layer of the lateral plate developing into a somatopleure. The reason why he did not observe this interesting reduplication of the somatopleure may be that his embryos did not live long enough. He stated that only the embryos which lived for a sufficiently long time, showed forelimb rudiments. Most of the embryos had probably not reached the age at which the forelimbs became clearly visible.

O. MANGOLD (1936) has also described the extirpation of the whole entoderm (albeit in a different way). He merely mentioned the development of one or two supernumerary forelimbs in the area of operation in some of his experimental animals, but without further explanation.

As far as I know, a similar operation has not been performed by other authors than BALINSKY and MANGOLD.

These observations induced me to make a further investigation of the factors determining the development of the two layers into which the lateral plate is divided by the formation of the coelomic cavity.

B. NORMAL DEVELOPMENT OF THE LATERAL PLATES.

The following short description of the origin and the displacements of the cell material of the lateral plates and the blood

island during the early development has been composed from data given by VOGT (1926 and 1929), BIJTEL (1929, 1930 and 1931), NAKAMURA (1940), YAMADA (1938) and my own experiments on vital staining. A detailed description of the latter will be given on p. 88-102.

From the researches of BIJTEL, VOGT, and other authors, we know that only the cranial part of the mesoderm, *viz.*, the first eleven or twelve segments, has been invaginated at the early neurula stage. A large part of the mesoderm of the caudal part of the trunk and the mesodermal cell-material of the tail has not yet been invaginated. It is still situated in the external cell layers surrounding the blastopore. This cell-material invaginates during the closure of the neural tube.

The mesoderm of the presumptive lateral plates lies ventrally and latero-ventrally to the blastopore at the late gastrula stage. As a result of the marked dorsal convergence, it forms, after invagination, first the ventro-lateral and lateral parts of the mesoderm. The medio-ventral and latero-ventral internal parts are occupied by the presumptive blood island. Latero-cranially to the latter, at the right and left sides of the mesoderm-free field, the cell-material of the presumptive heart rudiments is situated. (Cf. concerning the topographical subdivision of the marginal zone the chapter on Terminology on p. 57).

During the early neurula stages the notochord begins to separate, and soon forms a median column of cells, situated between the right and left mesoderm. The presumptive myotomes are bordered laterally by the material of the pronephros and the nephrogenous cord which forms a rather narrow strip of cells between the presumptive myotome and lateral plate mesoderm. Cf. fig. 35, d-f, zone e and f, p. 97. Later at the elder neurula stage, the presumptive lateral plate mesoderm also forms two rather narrow cell-strips, situated on either side of the entoderm mass. Cf. fig. 35, d and e, p. 97. Shortly before, and also during, the stretching of the embryo, the rudiment of the blood island changes greatly in form. The broad and rather short field, occupied in the neurula, becomes elongated, and finally forms a narrow strip of mesodermal cells in the ventral median line. Cf. fig. 35, f, p. 97.

In connection with these displacements of the cell material of the blood island, the adjacent mesoderm of the lateral plates extends in a ventral direction. In this way the lateral plates

cover the entoderm mass on the lateral and ventral sides in the young larva. Cf. fig. 35, f, p. 97.

After the mesoderm of the presumptive lateral plates has grown round the entoderm mass, the presumptive somatopleures and splanchnopleures are formed by the appearance of the coelomic cavities. The extending coelomic cavities meet each other in the dorsal and ventral median lines, and unite after the disappearance of the ventral mesentery. The peritoneal epithelium differentiates over the entire inner surface of the coelomic cavity.

After the formation of the neural tube, the cell material of the neural crests migrates ventrad, and forms the greater part of the corium and the interstitial mesenchyme. It also furnishes the chromatophores, which settle subepidermally along the dorsal side of the myotomes and nephrogenous cord and in the mesenchyme of the somatopleures.

Myoblasts, originating from the ventral parts of the myotomes, then migrate into the somatopleure. They form a very thin muscle layer, extending from the myotomes to some distance before the ventral median line. The medio-ventral parts of the somatopleures remain free from muscle fibres. This fact is characteristic of the region of fusion of the somatopleures and splanchnopleures of both sides.

In the cranial region of the trunk the forelimb rudiments differentiate in the dorsal part of the somatopleures. The first radially symmetrical thickening in the mesoderm grows out in caudal direction. Here the third, fourth and fifth spinal nerves innervate the forelimb muscles and the corresponding muscle plate segments of the somatopleure. In the middle segments of the trunk the spinal nerves innervate the myotomes, muscle plate segments and the skin. Finally, in the caudal segments, the corresponding spinal nerves also innervate the hind limbs, originating at a later developmental stage.

The differentiation of the splanchnopleure proceeds in a different way. As far as we know, all the cells of the presumptive splanchnopleure differentiate in situ. They form the mesenchyme and muscle cells of the intestinal wall. Nerve cells and nerves enter the splanchnopleure through the dorsal mesentery. The nerve cells form the nerve plexuses of the intestine, the nerves are both sympathetic and parasympathetic.

As we have seen, the development and differentiation of the

somatopleure and splanchnopleure differ widely; we can summarize the differences between the two layers in the following points:

- | | |
|--|--|
| Ia. The somatopleure contains a segmented muscle plate, which originates from the myotomes. | Ib. The splanchnopleure contains only scattered smooth muscle cells which are not yet numerous in the larval stages studied (up till stage 43 GLAESNER). |
| IIa. Large chromatophores are found scattered in the somatopleure. | IIb. Chromatophores are lacking in the splanchnopleure. |
| IIIa. In the somatopleure a characteristic forelimb rudiment develops. | IIIb. In the splanchnopleure no such local differentiations occur. |
| IVa. The mesenchyme of the subcutis is rather widemeshed and, therefore, the somatopleure is more voluminous than the splanchnopleure. | IVb. The splanchnopleure forms a rather compact, thin layer. |
| Va. The nerve supply is furnished by the spinal nerves. The forelimbs are innervated by the strongly developed third, fourth and fifth nerves. | Vb. The nerves in the splanchnopleure are very thin, and difficult to recognize. They are both sympathetic and parasympathetic. |

C. EXPERIMENTAL PART.

Series 1) The extirpation of the whole entoderm. Experiment and results.

As the entoderm and mesoderm in the cephalic region are not entirely separated from each other before the appearance of the neural folds, the operation could not be performed at an earlier stage. It was, therefore, done at the early neurula stage.

Embryos of *Triturus taeniatus* and, in some cases, of *Tr. alpestris* were used.

After the sterilisation, and the removal of the yolk membrane, the neurulae were transferred to small pits in the wax bottom of an operation dish, and turned with their ventral side upwards. With a glass needle the ectoderm and underlying mesoderm were

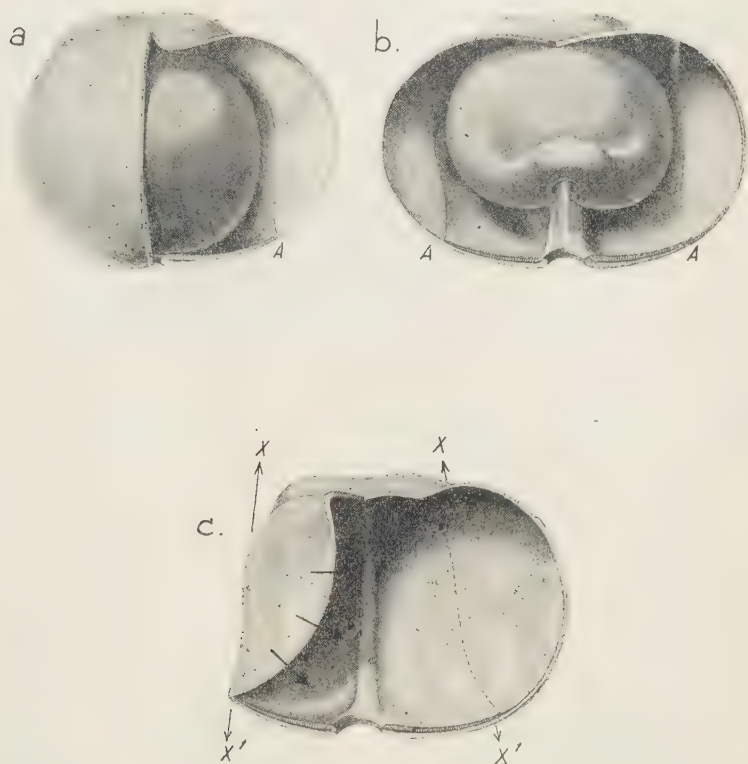


Fig. 1. Operation sketch of removal of whole entoderm at early neurula stage. a) Ectoderm and underlying mesoderm cut in ventral median line and separated from central entoderm on the left. Line A: Anterior border of invaginated mesoderm. b) Same preparation at other side and separation of caudal part of entoderm from chordo-mesoderm. c) Inner side of ecto-mesodermal envelope. Line X-X': Folding line of mesoderm-free ectoderm. On right side, ectoderm already folded inwards against inner surface of mesoderm. Enlargement $\pm 24\times$.

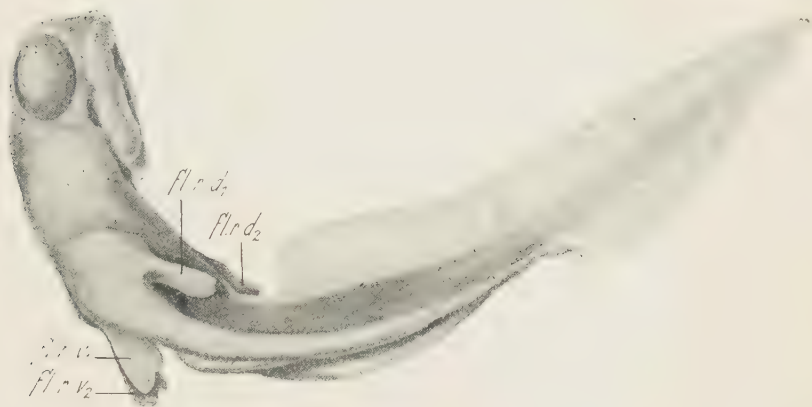


Fig. 1. Entoderm-free larva, *Tr. alpestris* 399, 20 days after operation. Head shows microcephalic development. Trunk flattened on ventral side. Reduplication of numbers of forelimb rudiments (fl. r.). Normal dorsal body and tail fins. Enlargement $\pm 25\times$.

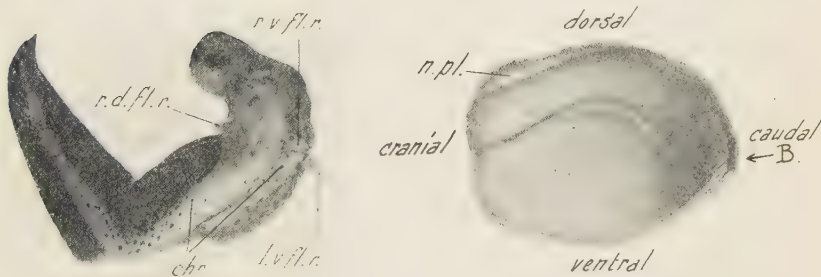


Fig. 2. Photograph of entoderm-free larva, *Tr. taeniatus* 369, 32 days after operation. Two ventral (r. v. fl. r., l. v. fl. r.) and one dorsal (r. d. fl. r.) forelimb rudiments are visible. Large chromatophores (chr.) in mesoderm of somatopleures and in forelimb rudiments on dorsal and ventral sides. Enlargement $15-20\times$.

Fig. 3. Ecto-mesodermal envelope of *Triturus* neurula some hours after removal of entoderm. Envelope folded together in medio-lateral direction, bringing inner sides of lateral plates into contact with each other. Enlargement $\pm 25\times$. B.: blastopore, n.pl.: neural plate.

cut in the ventral median line from the blastopore to the outer border of the cranial neural fold. Beginning at the blastopore, at one side the ectoderm and mesoderm were separated from the entoderm with the aid of two hair-loops. Pl. I, fig. 1a. After the same preparation on the other side, the caudal entoderm lay free, and could now be lifted of the dorsal chordo-mesodermal plate. Pl. I, fig. 1b. Then the whole entoderm was turned over from back to front, and gradually removed from the ecto-mesodermal envelope. Pl. I, fig. 1c.

The separation of the entoderm from the mesoderm presents difficulty in the pre-chordal and the stomodaeal regions. Only by a very careful method of operation is it possible to remove the entoderm completely without serious injury to the ectoderm and mesoderm of this part of the embryo. In the middle and caudal parts of the trunk the separation can be performed more easily without any injury to the other germ layers.

Entoderm cells left behind, could be easily removed after spreading out the ecto-mesodermal envelope. They were recognizable from their light colour.

The mesoderm-free parts of the ectoderm formed long strips, situated on both sides of the chordo-mesodermal plate after the spreading out of the ecto-mesodermal envelope. This ectoderm was folded inwards along the outer border of the mesoderm (line X-X', pl. I, fig. 1c) and soon attached itself, with its inner side, to the mesoderm. The large wound closed in a few hours by the strong tendency of the ectoderm to spread over the mesoderm.

The next day the neural tube had been formed. The caudal part of the trunk had been bent to the ventral side, and the middle trunk region had much been flattened owing to the absence of the entoderm. After the formation of the tail bud, the stretching of the embryo began. The differentiation of the various organs proceeded normally till the cells had consumed their yolk material, after which development ceased. Plate II, figure 1 and 2 show two larvae, reared for twenty and thirty-two days respectively, after the removal of the entoderm. The first shows the formation of the hand-plate, while in the older larva two or more finger-rudiments are already present.

The most striking deviation from the normal development in these entoderm-free animals was the presence of two pair of forelimbs. Their position is very interesting, one pair being situated

on the dorsal side of the body, the other on the ventral side, opposite the pair first mentioned (pl. II, fig. 1). By the spreading out of the ecto-mesodermal envelope its originally lateral parts will form the dorsal side of the embryo. The forelimbs normally originate in the dorsal part of the lateral plate. From these two facts we may conclude that the dorsal pair represents the normal forelimbs, whereas the ventral forelimbs are supernumerary.

In *Triturus alpestris* no. 399 (pl. II, fig. 1) all the limbs had the same degree of differentiation; they exhibited the first indication of the hand-plate. In other experimental animals, size and differentiation of the ventral limbs were inferior to those of the dorsal pair.

The greater part of the experimental animals of this series showed this redoubling of the number of forelimbs. Of the 14 animals reared, 8 showed two pairs of forelimbs; in one of these cases the supernumerary forelimbs were situated close against each other in the ventral median line. Each of the six remaining animals had only one pair of normal dorsal limbs. This different structure of the experimental animals will be discussed and accounted for later on. Cf. p. 20.

Externally it was already apparent that in many cases the head was abnormally developed. All the animals experimented upon were more or less microcephalic; they showed various intermediate stages between total cyclopia and an almost normal structure of the head.

The entoderm-free animals showed a complete absence of external gills. Although MANGOLD's animals (1936) had stretched out normally, they were reduced in length. My animals were also smaller than the controls of the same age, and frequently had a strongly curved or geniculated form.

Here follows a description of some representatives of this set of experiments.

Triturus taeniatus no. 369, operated 28/5 '42, fixed 29/6 '42. Cf. pl. II, fig. 2.

The animal was microcephalic. The single nasal pit had no communication with the small, but well differentiated ectodermal stomodaeal invagination (mouth cavity) with many tooth rudiments. The teeth were in close contact with some cartilages which belonged to the mandibular arch. Other cartilages of the visceral skeleton were lacking.

The neck region and the anterior part of the trunk had sharply bent in backward direction, with the result that the head made a right angle with the trunk. The anterior segments of the trunk were a little hydropic in their lateral parts, in which the coelomic cavities were much enlarged. The heart

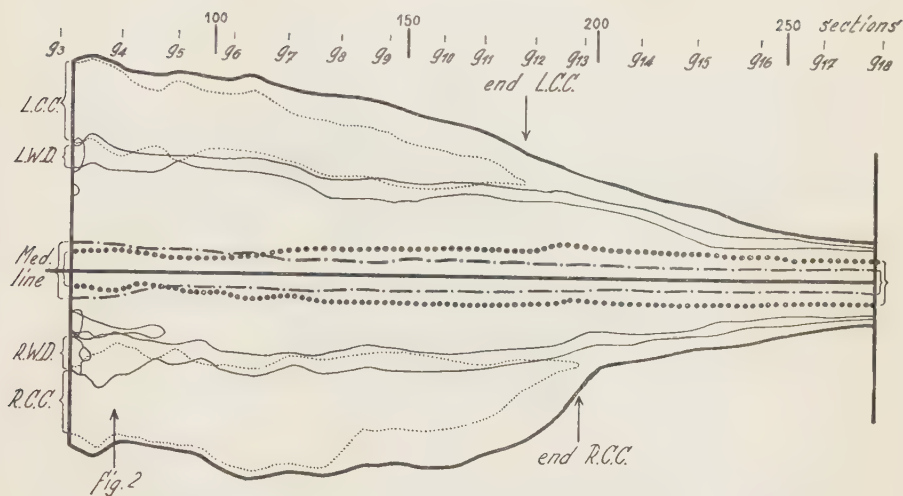


Fig. 1. Graphic reconstruction of trunk of *Tr. taeniatus* 369. Outlines of several organs projected on frontal plane (enlargement $50\times$ in medio-lateral direction, in cranio-caudal direction 1 section (8μ) = $\frac{1}{2}$ mm). Centres of notochord and spinal cord used for determination of median plane of body. — · — · — ·: Outline of spinal cord, ...: Outline of notochord, ———: Outline of Wolffian duct and pronephros, ———: Outline of body, ...: Outline of coelomic cavity, g_3 , etc.: Projected position of third spinal ganglion, etc.

rudiment was lacking. The dorsal and ventral forelimbs had already formed two or three toes. The dorsal and tail fins were well developed. The dorsal fin began as in normal animals, at some distance behind the forelimbs, the ventral tail fin immediately behind the point at which, in normal development, the ectodermal proctodaeum would be formed. The caudal part of the trunk was much flattened at the ventral side. The tail also made a right angle with the trunk in a dorsal direction.

The animal was almost bilateral-symmetrical (plane a-a' fig. 2). The right and left coelomic cavities were entirely separated from each other, and were situated in the most lateral portions of the trunk. In the anterior half of the trunk the coelomic cavities were well developed, whereas they had not been formed in the 13th to 18th segments. Cf. fig. 1. The Wolffian ducts ran medially to them, and opened to the exterior in the 18th segment anterior to the beginning of the ventral tail fin.

The cross section fig. 2 shows the triangular form of the trunk. The body was completely covered by the ectoderm, so that the mesoderm came nowhere to the surface. The mesenchyme had a hydropic character, the muscle fibres did not form compact myotomes or a continuous muscle plate. Nevertheless these parts could be clearly recognized. The coelomic cavities were situated on either side of the axial system of spinal cord, notochord and myotomes. The intestine was entirely absent.

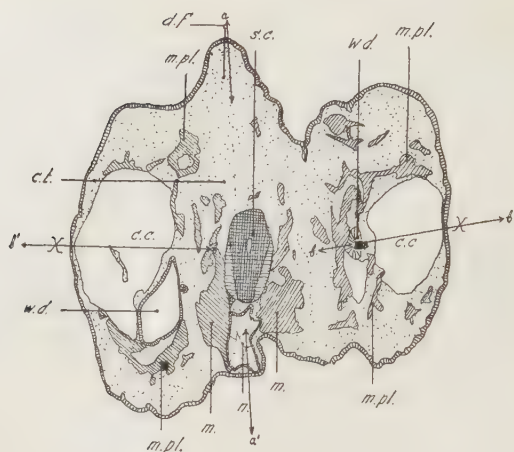


Fig. 2. Transverse section through cranial part of trunk of *Tr. taeniatus* 369. Point X: Point of transition between original outer and inner layer of lateral plate. Plane a-a': Plane of bilateral symmetry. Plane b-b': New plane of symmetry in lateral plate. Enlargement $\pm 60\times$. S.c.: spinal cord, n.: notochord, c.c.: coelomic cavity, m.: myotome, d.f.: dorsal fin, m.pl.: muscle plate, W.d.: Wolffian duct, c.t.: connective tissue.

The lateral plates, divided by the coelomic cavities into two layers, which lay dorsally and ventrally to them, showed a peculiar development. The two layers were quite identical in structure. Medially, where they were connected with the axial system, they had a great diameter. They decreased in thickness laterally, and at the most lateral point (X) they consisted of a very thin peritoneal layer only, which directly bordered upon the ectoderm. Both layers contained a well-developed muscle plate, consisting of characteristic cross-striated muscle fibres, derived from the myotomes. These plates also decreased in medio-lateral direction, and ended at some distance from the point X.

During the operation the ectoderm was folded inwards along the lateral border of the mesoderm.

From this mode of operation and the dorso-ventral flattening of the body of the embryo, it becomes clear that the boundary between the original outer and inner layer of the lateral plate corresponds to the point X. This is in agreement with the thinness of the lateral plate layers at this point and with the termination of the muscle plate at some distance from it. The body wall, dorsally to the coelomic cavity, has, therefore, developed from the outer layer of the lateral plate, whereas the ventral coelomic wall has derived from the inner layer.

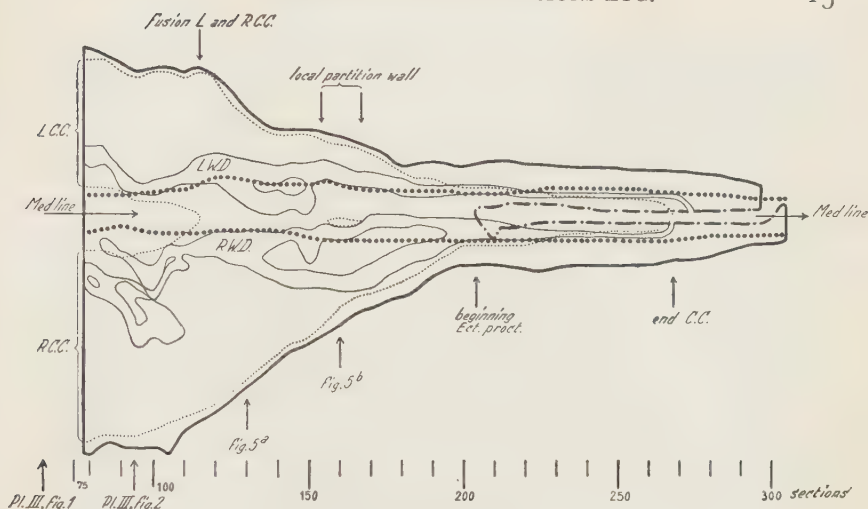


Fig. 3. Graphic reconstruction of trunk of *Tr. taeniatus* 384, fixed 31 days after operation. Projection on frontal plane (enlargement $40\times$ in medio-lateral direction, in cranio-caudal direction 1 section ($8\mu = 0,4\text{ mm}$). Outlines of organs indicated in same way as in fig. 1. — · — · — · —: Outline of ectodermal proctodaeum.

The position of the Wolffian duct was also interesting. In normal development, the pronephros, the Wolffian duct and the mesonephros are situated in the somatopleure. Here they lay at the base of both layers in a new plane of symmetry. This plane (b-b', fig. 2), situated in the lateral portions of the animals only, could be drawn through the Wolffian duct and the point X.

In this experimental animal, the Wolffian ducts were single throughout the whole trunk, whereas in other animals I found a partial reduplication of these ducts by longitudinal fission along a part of their course. Cf. fig. 3.

The photograph, pl. II, fig. 2, shows another interesting fact. The chromatophores, which are to be found in a normal larva in the somatopleure only, were present in both layers here. The ventral (inner) layer also contained a large number of scattered chromatophores.

The position and structure of the normal and supernumerary forelimbs will be recorded in the description in the next experimental animal.

Triturus taeniatus no. 384, operated 3/6 '42, fixed 4/7 '42.

The structure of the head was almost the same as in *Tr. taeniatus* no. 369, only the eyes were markedly synophthalmic. In the trunk a heart rudiment was absent. In the anterior part of the trunk the two coelomic cavities were separated from each other. They were situated more ventrally than in *Tr. taeniatus* no. 369. The planes of symmetry (b-b') of the right and left coelomic cavities and lateral plates did not coincide as was the case in the first animal, but formed an angle of about 100° - 120° . Cf. Pl. III, fig. 1. In more caudal segments where the coelomic cavities were situated more ventrally, this angle

decreased. Cf. Pl. III, fig. 2. The coelomic cavities soon united at some distance behind the end of the right pronephros, with the exception of a small stretch in the middle trunk region. There a very thin medial partition wall had been formed. Cf. fig. 5.

The graphic reconstruction of the trunk, Cf. fig. 3, shows the position of the coelomic cavities and Wolffian ducts. These ducts had fissured along part of their course. In the caudal part of the body there was an ectodermal proctodaeum invagination, into which the Wolffian ducts opened and which communicated with the outside through a small anal opening. The single coelomic cavity ended on a level with the aperture of the right Wolffian duct. The left coelomic cavity was regular in shape, and lay symmetrically in the lateral plate. The mesodermal layers between the coelomic cavity and the dorsal ectoderm, and between the coelomic cavity and the ventral side of the larva, were quite identical in structure. Both contained a well-developed muscle plate, lying close against the peritoneum and decreasing in thickness in lateral direction. It ended at some distance from the plane of symmetry (b-b'), where the mesoderm formed an extremely thin layer, consisting of a peritoneal membrane only, situated close against the ectoderm. Cf. pl. III, fig. 1. As in the former case, we can say with certainty that the point X in the plane of symmetry (b-b') corresponds to the region of transition between the outer and the inner layer of the lateral plate.

In the right ventral somatopleure two chromatophores were visible. Both layers contained a well-developed forelimb rudiment, sectioned through the proximal part of the humerus. As we could see in the living animal, Cf. pl. II, fig. 1, both limbs pointed in a latero-caudal direction; they were quite identical.

Pl. III, fig. 2 represents a cross section somewhat caudal to the first cross section, pl. III, fig. 1. It also shows the symmetrical structure of the right lateral plate of the embryo. The two coelomic cavities are situated more ventrally and at a shorter distance from each other than in pl. III, fig. 1. The right dorsal forelimb rudiment has been sectioned through the proximal end of the humerus and the caudal part of the scapula, whereas the right ventral forelimb has been cut through the middle of the humerus. Moreover, the photograph shows cross sections through the carpus and the base of the toes of the left forelimbs. The left ventral mesodermal plate contains three chromatophores, scattered among the mesenchyme cells and muscle fibres.

In order to get a clear view of the course of the nerves, innervating these forelimbs, I projected the nerves which ran through several sections, upon one cross section. Cf. fig. 4. The form and situation of the various organs were naturally not identical in the successive cross-sections. Thus, it was unavoidable that in the sketch the course of the nerves did not entirely correspond to their actual course through the body. The details being of less interest, however, the sketch sufficiently demonstrates the existing relations ¹⁾. ²⁾

¹⁾ On account of irregularities in the course of the nerves, it was difficult to trace all their branches. Only the largest could be recognized between the



Fig. 1. Photomicrograph of transverse section through cranial part of trunk of *Tr. taeniatus* 384. Enlargement 50 $\times$. Planes a-a', b-b' and point X as in fig. 2. B.l.: blood lacune, p.t.: pronephric tubules, d.som. and v.som.: dorsal and ventral somatopleure.

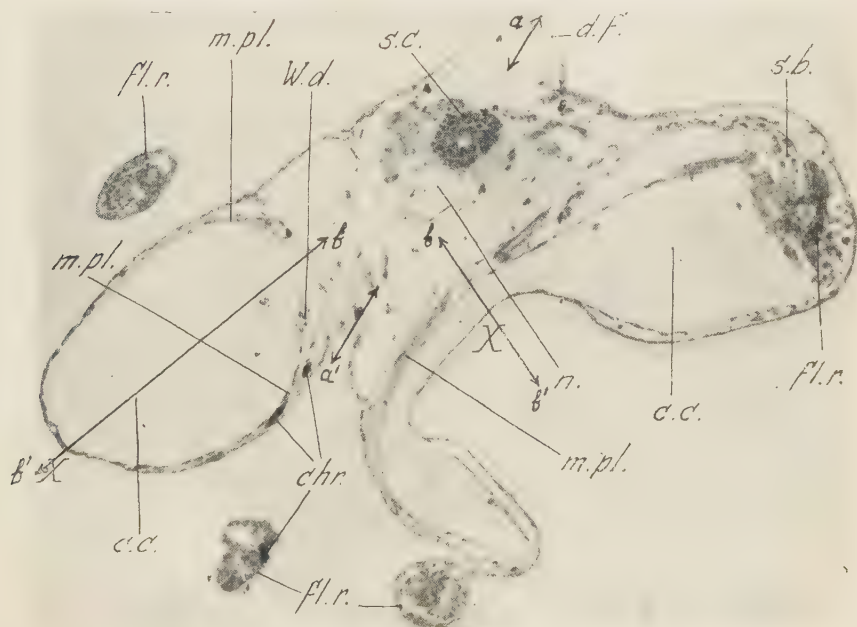


Fig. 2. Photomicrograph of transverse section of *Tr. taeniatus* 384, 30 sections behind the former, through left and right dorsal and ventral forelimb rudiments. Enlargement 50 $\times$. Planes a-a', b-b', and point X as in fig. 2.

forelimb and one to the dorsal muscle plate. On the right side, the fourth nerve ran along the notochord, and then bent to the lateral side. Arriving at the muscle plate, it split into two branches, one running to the dorsal and one to the ventral forelimb. Thus, one nerve innervates both the forelimbs on this side of the animal.

In the caudal part of *Tr. Taeniatius* no. 384 the two coelomic cavities had fused, and only the two normal somatopleures were

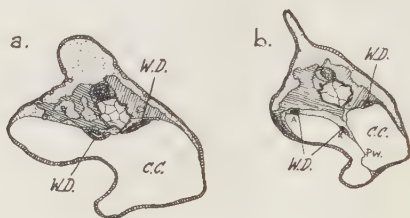


Fig. 5. a) Cross-section through middle trunk of *Tr. taeniatius* 384, caudally to fusion of coelomic cavities. b) Cross section at more caudal level, showing partition wall (p.w.) between coelomic cavities. Right Wolffian duct (A and A') split longitudinally. Enlargement 25 $\times$.

present. The fused inner layers had differentiated otherwise than in the cranial part of the trunk, where they remained separated from each other. They either formed only a very thin medial partition wall or they disappeared completely. Cf. fig. 5.

In this connection another small series of experiments, series 1', is of importance. Here – in deviation from series 1 – the inner sides of the presumptive lateral plates were laid against each other immediately after the removal of the entoderm. In these embryos the ectoderm covered the outer sides of the lateral plates, whereas the inner sides lay close against each other. The trunk of the larva showed no reduplication of organs or cell-layers.

Pl. II, fig. 3, gives a drawing of such an embryo a few hours after the removal of the entoderm. The medullary plate is beginning to fold up. The two mesodermal plates have fused over their entire inner surfaces.

Fig. 6a en b represent cross sections through the cranial and caudal parts of the trunk of animal *Tr. alpestris* no. 403. Only the outer layer of the lateral plate had been differentiated into a somatopleure with all its characteristics. Between the left and right Wolffian ducts, in the plane of symmetry, an irregular band of cells, formed from the inner layers of the lateral plates,

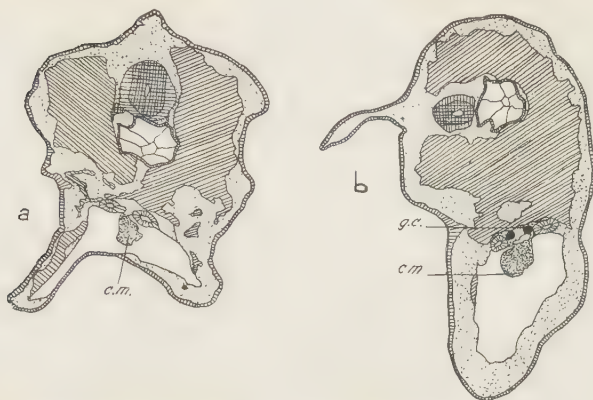


Fig. 6. Cross sections through trunk of entoderm-free larva *Tr. alpestris* 403, laterally compressed, showing entire absence of entoderm. Development of inner layers of lateral plates to a cell mass (c.m.), hanging freely in cocloemic cavity. This mass consists of vacuolated, peritoneal cells. Enlargement 50 $\times$. G.c.: germ cell.

hung down in the coelomic cavity. It consisted of very irregular vacuolated cells, the nature of which was difficult to determine, as they formed a degenerating mass. They bore most resemblance to hydropic epithelial cells.

Discussion.

1) *The development of the lateral plate.*

The dorsal and ventral layer of the lateral plate of the entoderm-free animals which have been flattened dorso-ventrally, resemble each other on the following points: 1) The strong development of the mesenchyme and the thickness of the layer itself. 2) The presence of a muscle plate, the fibres of which must have derived from the myotomes. 3) The position of the Wolffian duct in a new plane of symmetry, to be drawn in the lateral plate, and its tendency to reduplicate. 4) The development of forelimbs on the same level in both layers. 5) The innervation of these forelimbs and muscle-plates by the same spinal nerves. 6) The presence of large chromatophores.

Comparing these points of resemblance with the characteristics of the somatopleure and splanchnopleure, cf. p. 10, we find complete agreement with the former. The spinal nerves and the chromatophores had penetrated into the dorsal as well as into the ventral layer. Especially this fact gives additional support to the view that in these animals the two layers of the lateral plate have differentiated into somatopleures.

In other animals, such as *Tr. taeniatum* no. 384, only the cranial part of the trunk, which has been flattened dorsoventrally, shows the reduplication of the somatopleures. On the other hand, in the caudal part only the outer layers of the lateral plates have formed somatopleures.

In order to understand the different organisation of the cranial and the caudal parts of the body, we have to consider the development of the inner layers of the lateral plates. In pl. III. fig. 1, the ectoderm remains at a fairly great distance from the notochord in the ventral median line. When we compare this situation with that in *Tr. taeniatum* no. 369 (fig. 2, p. 14), in which the ectoderm covers the ventral side of the notochord, a marked difference is to be seen.

Considering the method of operation and the first development of the embryos, we may explain the situation in *Tr. taeniatum* no. 384 as follows: During the closure of the large wound the inner side of the right and left mesodermal plates have partly fused in the median line, ventrally to the notochord. The ectoderm could cover and separate the two mesodermal plates only along the free inner surfaces. During the formation of the coelomic cavities the fused proximal parts of the ventral (inner) layers of the right and left lateral plates were under conditions differing from those of the distal parts, these not being fused; the former have not been covered by the ectoderm. Posteriorly the rate of fusion of the ventral layers increases. In the caudal half of the body the two mesodermal plates have united over their whole breadth; the ectoderm, therefore, covers only the outer layers here. Thus, in the caudal part of the body the embryos are flattened medio-laterally. They show the same structure as the animals of the experimental series 1', described on p. 18. In that series, namely, the inner sides of the lateral plates came into contact with each other immediately after the removal of the entoderm. In these embryos the fused inner layers first form a median partition-wall. This afterwards loses its communication with the somatopleures in the ventral median line and hangs down like an irregular tangle of cells in the coelomic cavity. As no muscle and interstitial cells have differentiated in this mass, but only epithelial cells have formed, it is evident that we have to do neither with a somatopleure nor with a splanchnopleure. It is obvious that we should think of an independent mode of development of the inner layers of the lateral plates.

In order to explain these results, the following hypothesis concerning the development of the layers of the lateral plate into either a somatopleure or a splanchnopleure, is advanced here: In the independent state, the two layers have the same developmental potencies. In this state, where there is no influence of either the ectoderm or entoderm, they form merely a thin epithelium. Under the influence of the ectoderm, however, they differentiate into somatopleures, whereas the entoderm determines them to become splanchnopleures.

By this hypothesis, we can also give a satisfactory explanation of the fact that only 8 of the 14 experimental animals of series 1 show a redoubling of the number of forelimbs. Only those parts of the inner layer of the lateral plate, which have been covered by the ectoderm since the early neurula stage, are determined to a somatopleure. The other parts which were not in contact with the ectoderm differentiated independently.

Embryos, in which the whole inner side of the mesoderm has been covered by the ectoderm, show a differentiation of the inner layer of the lateral plate into a somatopleure over its whole length (3 of the 14 animals operated upon). Other embryos, in which the inner layers of the lateral plate have fused over the whole inner surface from the early neurula stage, do not show this double formation of somatopleures, forelimbs, etc. (4 of the 14 animals operated upon). Between these two extremes there are several intermediate stages in which, for instance, the somatopleures have been reduplicated only in the cranial half of the trunk (7 of the 14 animals operated upon). Two animals do not show reduplication of the forelimbs, this being probably prevented by hydropy of the trunk.

2) *The microcephaly of the entoderm-free animals.*

The separation of the germ layers in the pre-chordal plate had not yet been ended at the stage of operation (the early neurula stage). So it was unavoidable that, together with the removal of the whole entoderm, parts of the pre-chordal plate were also extirpated. This accounts, probably, for the microcephalic and cyclopic malformations (ADELMANN, 1936) and LEHMANN (1938a). MANGOLD (1936) and BALINSKY (1939) also observed these forms of microcephaly in their entoderm-free animals.

3) *The formation of the ectodermal mouth cavity.*

As STROËR (1933) already proved, the formation of the ectodermal mouth cavity is chiefly dependent on the oral entoderm. BALINSKY (1939 a and b) found an ectodermal stomodaeum invagination also in his entoderm-free ani-

imals. His further investigations in 1939 and 1940 brought to light that an ectodermal stomodaeum invagination had not formed, after the oral entoderm had been removed at the late gastrula stages. When the same operation had been performed, however, at the early neurula stage, an invagination had formed in a large number of cases. The determination of the mouth ectoderm begins in the late gastrula under the influence of the oral entoderm. It is evident that a contact of very short duration (from the late gastrula till the early neurula) between the two layers is sufficient for the ectoderm to acquire weak differentiation tendencies (Cf. p. 32 and Raven (1938)). On the other hand, a long contact (till stage 18-21 HARRISON) between the two layers is necessary for the complete autonomic differentiation of the ectodermal mouth cavity. My experiments at the early neurula stage confirm BALINSKY's conclusions. He obtained a formation of an ectodermal stomodaeum in 50% of the cases (operated at stage 15 HARRISON); I, however, found in 13 out of 14 animals such a formation. This difference is only gradual, and may be accounted for by the use of eggs of different cultures.

The possibility that oral entoderm cells have been left behind in my experimental animals, so that the induction of the mouth ectoderm has continued, may be dismissed, as the entoderm cells were carefully removed during the operation.

From these experiments we may conclude that the induction of the ectodermal mouth-plate begins in the later phases of the gastrulation. Differentiation tendencies to the formation of this mouth-plate can manifest themselves only after an induction period from the end of the gastrulation till the early neurula stage. BALINSKY's experiments (1940) further indicate that the other organs of the head also exert an important influence upon the differentiation of the "weakly" determined stomodaeum invagination. In STROËR's experiments the power of self-differentiation did not appear until a much older stage. He transplanted the mouth ectoderm into the flank. The difference between his results and my own may therefore be accounted for by the absence of local stimulating factors (in the head), and by the presence of counteracting factors (in the flank), by which the weak differentiation tendencies are suppressed. It is also possible, as BALINSKY suggested, that in STROËR's experiments, the transplants may have been resorbed.

4) *The formation of the teeth.*

I never found tooth rudiments in the absence of any adjacent cartilages in any of my animals operated upon. There must, therefore, be some relation between the cartilages of the visceral skeleton and the formation of teeth. STROËR (1933) has also mentioned the possibility of such a relation. The development of the teeth in the stomodaeum ectoderm is perhaps brought about by secondary induction from certain neighbouring cartilages of the visceral skeleton. WOERDEMAN (1943) came to the same conclusion from his xenoplastic mouth-ectoderm transplantations between *Triturus* and *Ambystoma*.

5) *The development of the visceral skeleton and the external gills.*

The visceral skeleton is represented in the entoderm-free animals by the cartilages of the mandibular arch only. BALINSKY (1939) and MANGOLD (1936) also stated this fact. The absence of external gills in my entoderm-free animals is in striking agreement with the investigations of EKMAN (1913), HARRISON

(1921), SEVERINGHAUS (1930) and ICHIKAWA (1933 and 1935), which show that the entoderm induces the external as well as the internal gills. The experimental animals of MANGOLD (1936) and BALINSKY (1939) have the same defects. It is evident that 1) the formation and determination of the gills and the visceral skeleton are influenced by the head entoderm, 2) the irregularities of the cartilages of the mandibular arch may be related to the microcephalic development of the head, 3) the latter phenomenon in its turn influences the development of the mouth cavity (BALINSKY, (1940)).

6) *Development of heart and blood island.*

MANGOLD (1936) stated that in his animals the heart had often developed rudimentarily, while the blood islands were also lacking for the greater part. Only in the caudal part of the body were some blood cells found. BALINSKY (1939a) could not find any indication either of a heart or of the blood island.

Even in the oldest of my animals, reared for 32 days after operation, there was no indication of a heart, so that I am in a position to confirm BALINSKY's statement. The possibility of a dedifferentiation of the original rudiment must, however, still be investigated. Hence it may be concluded that the entoderm also has an important influence in the formation of the heart.

As regards the blood islands and blood cells, I cannot confirm BALINSKY's opinion. Some of my animals showed degenerating blood cells in the coelomic cavity and in the mesenchyme of the ventral part of the trunk. Mrs. BRAUN-ENGELHARDT (1940) found that in the absence of the entoderm the mesodermal blood cells began to differentiate, but soon degenerated, and finally disintegrated completely. In HOLTFRETER's (1938) and YAMADA's (1937 and 1940) explants of the mesoderm of the presumptive blood islands, made at early neurula stages, numerous blood cells differentiated in the absence of the entoderm. As to the determination of the blood cells in the medio-ventral mesoderm during gastrulation, nothing is known. As the blood island originates from the internal blastoporal lip which is already in contact with the entoderm mass at the blastula stage, a very early determination of the blood cells by the entoderm is quite possible. The entoderm, however, seems necessary for the complete differentiation of the blood cells; moreover, the blood circulation may also have an influence.

Series 2. Entoderm-free animals, reared upon a collodion membrane.

Experiment and results.

This series of experiments gives additional evidence of the independent development of the inner layer of the lateral plate in the absence of inducing influences from the ectoderm or entoderm. For this purpose, I reared the outspread ecto-mesodermal envelope upon an indifferent substrate. To this end I used collodion. This is soluble in a mixture of equal parts 100% alcohol and ether (water-free) so that the substrate can be readily removed without injury to the adjacent tissue, when microscopic preparations are made. Moreover, it was to be expected that

pieces of thin collodion membrane ¹⁾ would be easily permeable by dissolved oxygen, and in this way ensure a normal respiration of the tissues. The collodion proved to be a happy choice also in other respects, for the inner side of the ecto-mesodermal envelope rapidly attached itself firmly to this substrate.

The operation was performed at the early neurula stage. The removal of the entoderm was made in the usual way (cf. p. 10).

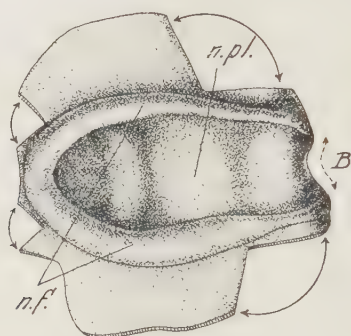


Fig. 7. Entoderm-free ecto-mesodermal envelope of *Tr. alpestris* 413, spread out upon collodion membrane after incising in latero-caudal and latero-cranial angles, some hours after operation. The cut edges of ectoderm and mesoderm have been hatched. Enlargement $\pm 30\times$. N.f.: neural fold.

Then the ecto-mesodermal envelope was spread out by making four radial incisions, *viz.*, a deep one to the right and left of the caudal part of the medullary plate and a small one in the head ectoderm on either side of the median plane. Cf. fig. 7. Then I placed the ecto-mesoderm layer with its inner side on the collodion and pressed it gently against the membrane. It quickly adhered to the collodion over its whole inner surface, and developed quite normally.

The next day the neural tube had closed, and the eyes became visible. Cf. fig. 8. The ectoderm and underlying mesoderm grew out over a large area, and the embryos turned into large flat

¹⁾ This collodion membrane was made by pouring a solution of collodion (in alcohol/ether) over a flat and polished glass-plate. After drying the membrane was removed from the glass and washed in streaming water for several hours to remove the last traces of the solvent.

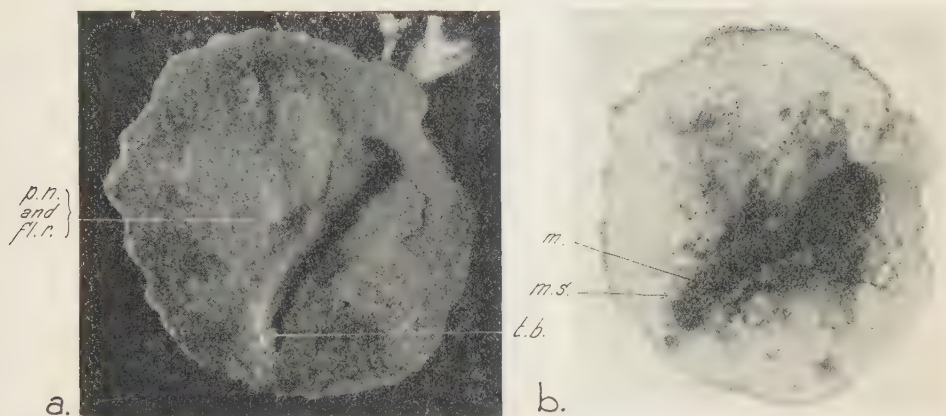


Fig. 1. a) *Tr. alpestris* 412, cultivated upon collodion membrane; fixed 7 days after operation; reflected light. b) Same embryo, transmitted light. Enlargement $\pm 20\times$. M.s.: myosepta.

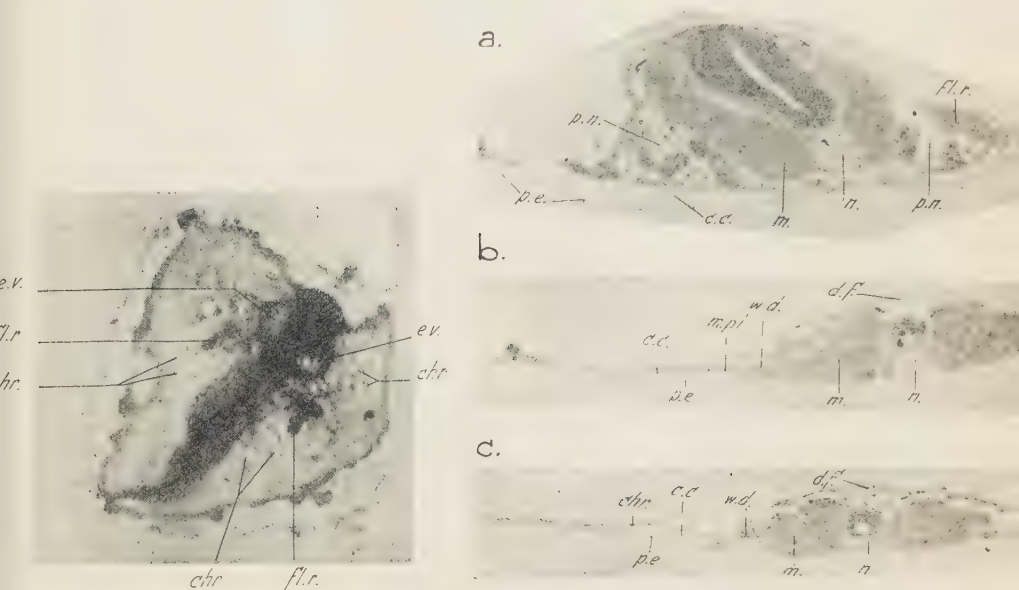


Fig. 2. Photograph of *Tr. alpestris* 413, cultivated upon collodion membrane for 20 days. Margins of thin ectodermal layer show signs of degeneration. Enlargement $\pm 20\times$. E.v.: ear vesicle.

Fig. 3. *Tr. alpestris* 411, cultivated for 23 days upon collodion membrane. Photomicrographs of cross sections through anterior, middle and posterior trunk regions, respectively. Enlargement $\pm 60\times$. P.n.: pronephros, p.e.: peritoneal epithelium.

discs. In these animals, the outside of the mesoderm is covered by the ectoderm, whereas the whole inner side of the chordomesodermal plate lies upon the collodion membrane.

The differentiation of these embryos proceeded almost normally. In the embryo of pl. IV, fig. 1 the outgrowth of the ectoderm and mesoderm reached its maximum. Up to its outer mar-

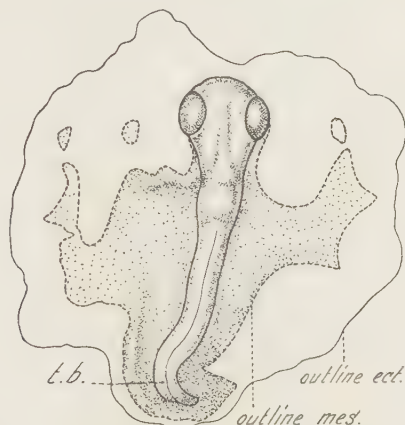


Fig. 8. *Tr. alpestris* 418, one day after operation. Ectoderm and mesoderm grown out upon collodion membrane over a large area. —: Outline of ectoderm, - - - - -: Outline of mesoderm. Enlargement $\pm 30\times$. T.b.: tail-bud.

gin the ectoderm formed a well-differentiated epithelium. In pl. IV, fig. 1a, the differentiation of the head and tail are clearly recognizable. The embryo also shows the first indications of the forelimb and pronephros rudiments in the form of a slight thickening of the underlying mesoderm. Pl. IV, fig. 1b clearly shows the differentiation of the myotomes.

In *Tr. alpestris* no. 413, pl. IV, fig. 2, most of the embryonic organs had formed completely. The photograph shows a microcephalic head (the dark anterior part), probably with cyclopic or synophthalmic eyes and fused naseal pits. The two large ear vesicles are visible through the ectoderm in the caudal part of the head. Caudally to them, the forelimb rudiments may be seen, in which the cartilages of the humerus and scapula are already visible. In the trunk the spinal cord and notochord may be recognized as a single dark band between the two ranges of myotomes. At its outer margin the ectoderm shows symptoms of



Fig. 9. Sketch of cross section through pronephric region of *Tr. alpestris* 413, cultivated upon collodion membrane for 23 days. Enlargement 50 $\times$. N.e.: nucleus of peritoneal epithelium cell.

degeneration and destruction, probably by lack of food, because of the cells having completely consumed their yolk material.

During several days these embryos showed rhythmical swimming movements which often became so vigorous that the embryos freed themselves from the collodion membrane and swam about freely. This process began with the raising of the head. As a result of the violent movements an increasing portion of the embryo gradually loosened from the membrane. The lateral ectoderm and mesoderm folded inwards, and their outer margins grew together, enclosing a large cavity. Thus, an almost normal animal was produced, except for the lack of the entoderm.

These facts clearly demonstrate the vitality and normal development of these experimental animals. They are a strong argument against the assumption of any possible harmful influence of the collodion upon the embryo. Further evidence against this assumption is afforded by the following microscopical analysis of the larvae.

As the photomicrographs, pl. IV, fig. 3, show, the differentiation of the organs, such as: spinal cord, notochord, myotomes, pronephros, somatopleure with muscle-plate and forelimb rudiment, is very good. They have an almost normal position. In the first section the right and left pronephroi and the left forelimb rudiment may be seen, in the second and third the Wolffian ducts are cut. The second photomicrograph shows the right muscle-plate.

The animal contained a large slit-like coelomic cavity which was apparently situated between the outer layer of the lateral plate and the collodion membrane. A microscopic examination, however, evinced that the inner layer of the lateral plate was

also present, in the form of a very thin epithelium, covering the collodion membrane. It consisted of very large flat peritoneal cells, and was so thin that it could hardly be seen on the photomicrographs. By a comparison with the diagrammatic sketch, fig. 9, however, it can also be distinguished in the photographs.

From this development it is evident that the outer layer of the lateral plate, which is covered by the ectoderm, has differentiated into a normal somatopleure with forelimb rudiment, muscle-plate, etc. Moreover, the pronephros is situated in this layer, and there are large chromatophores (cf. pl. IV, figs. 2 and 3c and fig. 9) among the scattered mesenchyme cells.

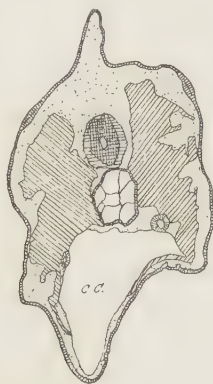


Fig. 10. Cross section through middle trunk region of *Tr. alpestris* 410, cultivated upon collodion membrane for three weeks; it freed itself of membrane some days before fixation. Somatopleures grown together, enclosing single coelomic cavity. Remains of inner lateral plate layers are absent. Enlargement 50 $\times$.

No smooth muscle cells and mesenchyme cells but only peritoneal cells are present in the inner layer of the lateral plate. We may conclude from these facts that it has not differentiated into a splanchnopleure. We have evidently to do here with the independent state of the inner layer of the lateral plate.

Fig. 10 shows a cross section of an embryo which had freed itself from the collodion membrane, as described above. The axial organs, Wolffian ducts and somatopleures with muscle-plates, have differentiated normally. The central cavity, situated between the left and the right somatopleure, corresponds to the fused coelomic cavities and may be considered as such. Any trace of a splanchnopleure or thin medial epithelium is lacking. This is

to be expected, when we bear in mind the history of these animals; the inner layer, being a very thin epithelium which covered the collodion membrane, was torn off and remained behind, when the embryo freed itself from the collodion membrane.

Discussion.

These animals demonstrate the peculiar differentiation of the inner layer of the lateral plate in the absence of adjacent ectoderm or entoderm. Just as in the preceding experimental series, the inner layer has formed a thin peritoneal epithelium only, without any differentiation of mesenchyme and muscle cells.

It is possible that the presumptive muscle and mesenchyme cells did not differentiate, and died at an early developmental stage, the peritoneal cells only remaining. I, however, never found degenerating cells in the inner layer, while remains of disintegrated cells are likewise absent. The vitality of the animals and the good differentiation of the cells are further evidence against the assumption of a possible harmful influence of the collodion upon the embryo.

The very small number of peritoneal cells in the inner layer of these experimental animals raises the question as to whether the cells of the splanchnopleure might have chiefly an extra-regional origin. As far as we know, there are, however, no indications of such a migration in the normal development. It seems probable, therefore, that all the cells of the inner layer of the lateral plate have differentiated into peritoneal cells here, and that this state represents their independent development.

Series 3. Medio-lateral reversal of the presumptive lateral plate mesoderm.

Experiment and results.

In this last series of experiments the medio-lateral axis of the mesoderm of the presumptive lateral plate was reversed at the early neurula stage by means of a reciprocal transplantation.

The operation was carried out in the following way. Cf. fig. 11. Two neurulae of *Tr. alpestris* of the same age were turned upon their sides, one with its right and the other with its left side upwards. Then a fairly large rectangular area of the ectoderm, covering a.o. the presumptive mesoderm of the lateral plate, was cut along the cranial, ventral and caudal sides in the exposed flank.



Fig. 1. Photograph of *Tr. alpestris* 405, reared for 27 days after medio-lateral grafting of presumptive lateral plate mesoderm in anterior part of right flank (pronephros and forelimb region). Embryo entirely normal. Enlargement $\pm 4\times$.



Fig. 2. a) Photomicrograph of cross section through anterior part of trunk of *Tr. alpestris* 406, reared for 27 days after medio-lateral grafting of presumptive lateral plate mesoderm in anterior part of right flank. b) Photomicrograph of cross section through posterior part of trunk of *Tr. alpestris* 408, reared for 26 days after same operation in caudal part of right flank. Enlargement $60\times$. S.b.: shoulder blade, spl.: splanchopleure, i.: intestine and l.: liver.

By careful preparation it was separated from the underlying mesoderm and folded back towards the dorsal side. Then the exposed mesoderm was cut along its four sides and loosened from the entoderm. The second neurula was prepared in the same way in the corresponding area at the other, exposed, side of the body. Care was taken to place the graft in such a way that the dorso-ventral and antero-posterior directions of the graft coincided

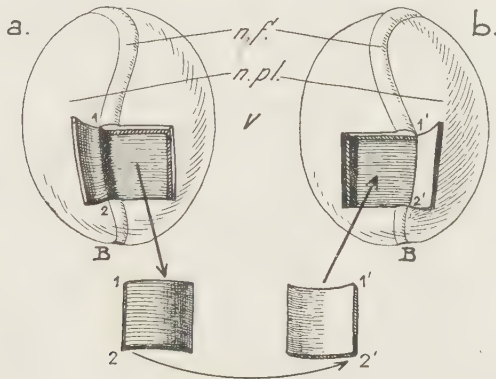


Fig. 11. Diagram of transplantation of presumptive lateral plate mesoderm from right flank of neurula a into left flank of neurula b. Dorso-dorsal, cranio-cranial, *medio-lateral* transplantation. Enlargement $\pm 20\times$.

with those of the host, whereas the medio-lateral axis was turned. This was achieved by transplanting the graft, e.g., from the right flank of neurula A to the left flank of neurula B without turning it round one of its axes. Cf. fig. 11. Then the ectoderm was folded back. The original outer side of the lateral plate now lay against the entoderm and the inner side against the ectoderm. In 5 transplantations the anterior part of the lateral plate was transplanted and in 4 cases a more posterior part was taken.

The embryos developed quite normally, and not a single deviation from the normal course of development was observed. The experimental animal, *Tr. alpestris* 405, cf. pl. V, fig. 1, operated upon in the right flank in the anterior part of the trunk (forelimb and pronephros region), showed an entirely normal and symmetrical external form. The photomicrograph, pl. V, fig. 2a, shows the symmetrical development of the right and left prone-

phroi and somatopleure with muscle-plate and scapula. The symmetrical and normal development of the splanchnopleures on both sides of the intestine and liver is also clear. The second photomicrograph, pl. V, fig. 2b, demonstrates the perfectly symmetrical position and structure of the lateral plates.

Discussion.

These experiments prove that the medio-lateral reversal of the lateral plate does not influence the formation of the somatopleure and splanchnopleure. One layer, lying against the ectoderm, always differentiates into a somatopleure and the other, covering the entoderm, always forms a splanchnopleure.¹⁾ Thus, in the early neurula stage, the lateral plate has not yet been determined in medio-lateral direction. The presumptive cell material of the two layers is still interchangeable without the appearance of abnormalities. The determination of the medio-lateral axis occurs at an older stage, probably between the neurula and the early tail-bud stage, at any rate before the differentiation of the somatopleure (in other words, before the earliest indication of the pronephros and forelimb rudiments and the beginning of the migration of myoblasts and chromatophores into the somatopleure).

D. DISCUSSION OF THE EXPERIMENTS IN PART I.

The development and determination of the head, ectodermal mouth cavity, teeth, visceral skeleton, blood island and heart have already been discussed on p. 21-23.

Before proceeding to discuss the formation of the cell-layers of the lateral plate, I will first consider the formation of the coelomic cavity. The caudal factors determining this process are still totally unknown.

YAMADA (1937) analysed the inductive influence of the notochord upon the various parts of the mesodermal envelope. He supposes that an induction field exists, emanating from the notochord and extending through the entire mesodermal envelope. Unfortunately he does not consider the formation of the lateral plate, so that any influence of the axial organs upon the formation of the coelomic cavity remains an open question.

Since only the anterior segments of the mesoderm have been invaginated at the early neurula stage, the entoderm could have influenced only the anterior segments of the trunk mesoderm. Some of my entoderm-free animals showed reduced, and others lacked, the coelomic cavities in the caudal part of the trunk. Cf. fig. 1, p. 13. In other experimental animals of the same series,

¹⁾ A migration of the cells of the outer and inner layer to their original positions may be excluded. I have never found such a phenomenon described in the literature.

however, the coelomic cavities had also been formed in the posterior part of the trunk although they were often more or less reduced. An influence of the entoderm on the development of the coelomic cavities seems, therefore, doubtful.

Surveying the various experimental series, it is evident that in this experimental analysis one link is missing: *viz.*, the independent development of the outer layer has not yet been investigated.¹⁾

Regarding the entire interchangeability of the outer and inner layers of the lateral plate, however, it is very probable that the outer layer will also differentiate into a thin epithelium, if it is influenced neither by the ectoderm nor by the entoderm.

Summarizing the whole series of experiments, we are able to state the following facts: The inner layer of the lateral plate, in contact with the entoderm, forms a splanchnopleure, whereas the outer layer, covered by the ectoderm, differentiates into a somatopleure (normal development). The inner layer of the lateral plate, covered by the ectoderm from the neurula stage, develops into a somatopleure. Without any influence of either ectoderm or entoderm it differentiates independently, *viz.*, into a thin peritoneal epithelium. At the early neurula stage the presumptive cell material of the outer and inner layers can still be interchanged without the appearance of abnormalities. It is probable that the independent development of the outer layer may also give rise to a similar thin epithelium.

Thus I am justified in concluding that the hypothesis advanced, cf. p. 21, on the determination of the two layers of the lateral plate, is confirmed by the results of my experiments.

¹⁾ Experiments of this nature have not yet been performed owing to technical difficulties. The removal of a large area of ectoderm is easy enough, but it is very difficult to prevent the overgrowth of the exposed mesoderm by the extending ectoderm. An attachment of the outside of the lateral plate to a collodion membrane over a rather large area meets with other difficulties. When the piece of collodion is large, the embryo frees itself very rapidly from the collodion after the appearance of the first movements and the wound closes in a few hours. A very small piece of collodion might perhaps be able to serve the purpose desired. (It may only cover the wound field and the surrounding margin of the ectoderm, and the embryo must be able to carry it along when swimming.) A reversal of the presumptive lateral plate mesoderm, followed by the removal of the entoderm and the outspread of the ectomesodermal envelope over a collodion membrane, may also lead to the same result.

Concerning the development and the mode of determination of the forelimb rudiments we may refer to the papers of HARRISON (1918), BALINSKY (1925-1937), FILATOW (1930-1933), ROTMANN (1931 and 1933), POLEŽAJEW (1938 and 1939), RUUD (1929) and others.

The problem of what organ determines the forelimb, is still unsolved. From the numerous transplantation experiments it has become probable that no single organ rudiment may be considered as the induction centre of the forelimb. Presumably many influences from the environment together determine the localization of the forelimb rudiment (e.g. from the pronephros, muscle-plate, lateral plate, etc.). These organs had an entirely symmetrical position in or with regard to the two somatopleures in the entoderm-free animals. This may explain why the normal and the supernumerary forelimbs were formed at the same level in the two somatopleures and why they showed an entirely identical structure.

In these experimental animals, the inner side of the lateral plate is covered by ectoderm, which, normally, would have formed the skin of the ventral side of the embryo and which would have had nothing to do with the formation of the forelimb. Notwithstanding this, normally structured forelimbs have been formed in the inner layers (supernumerary). Evidently the formation of these supernumerary forelimbs cannot be accounted for by the assumption of a local induction from the ectoderm upon the lateral plate mesoderm. The mesoderm must give the local impulse to the formation of the forelimb.

According to RAVEN (1938), we may distinguish "reactive powers" and "differentiation tendencies" to a definite development of a group of cells. "Reactive powers" are weak active potencies, which, however, cannot express themselves without further inductive stimuli. On the other hand, "differentiation tendencies" are stronger active potencies which, however, can express themselves without any further influence. According to RAVEN, the reactive powers will pass into differentiation tendencies in the course of the determination process.

BALINSKY's experiments on limb induction have proved that the whole lateral plate at the tail-bud stage has reactive powers to limb formation, perhaps even weak limb tendencies. In my experiments, the layers of the lateral plate do not possess differentiation tendencies (possibly neither any reactive power) to

somatopleure or limb formation without preliminary action of the ectoderm. For there are no indications to somatopleure and limb formation in the independent state. Therefore, the ectoderm primarily excites the reactive powers and perhaps weak differentiation tendencies to somatopleure and limb formation in the whole lateral plate. The tendencies to limb formation are, however, still weak, and can easily be dominated and suppressed by tendencies to somatopleure formation. The mesoderm invigorates the tendencies to limb formation locally, and determines the limb rudiment definitively. Both inductive influences, the general influence of the ectoderm and the localized action of the mesoderm, are necessary.

Concerning the character of these influences there are two possibilities: 1) The inductive influences of ectoderm and mesoderm may simply be summed up; they are of the same nature. The inductive influences from the mesoderm only – without the primary impulse of the ectoderm – are, however, too weak to reinforce the reactive powers to limb or lateral plate formation to such an extent that they can cross the threshold between reactive power and differentiation tendency. The preceding awakening of reactive powers by the ectoderm cannot be spared (summation process). 2) We have not to do with a simple summation of the two inductive actions, but with a succession of two influences, which differ in qualitative respect. There is a difference between the characters of the two inductive processes and probably also between the character of the reactive powers and that of the differentiation tendencies. (Compare the determination of the germ ridges in Part III, p. 177.) We cannot yet decide between these two possibilities.

In the course of the development the limb tendencies are excited or intensified in the future limb regions and suppressed in the other parts of the flank, where the somatopleure tendencies become stronger. The region with reactive power (perhaps even with weak differentiation tendencies) to limb formation, originally occupying the whole flank, successively shrivels up to the area of the limb fields proper during the development of the embryo. This fact can be explained only by the admission of the presence of two counteracting influences. We may suppose, therefore, that secondarily the ectoderm suppresses the limb tendencies while activating the somatopleure tendencies. On the contrary, the mesoderm locally intensifies the limb ten-

dencies, whereby the somatopleure tendencies decrease in that region. The apparent contradiction between the primary and the secondary actions of the ectoderm may be explained as follows: The primary action may be nothing more than a general increase of the metabolic activity. Thereby, reactive powers to several organ-formations are excited. On the other hand, the secondary action is a specific one, and forms specific differentiation tendencies.

The development of supernumerary forelimbs from the original inner layer of the lateral plate proves that this part of the mesoderm also has limb potencies. The inner layer of the lateral plate does not present any indication of the potency to forelimb formation in the independent state. From the latter fact we are forced to conclude that we have to do here with passive limb potencies.

Concerning the significance of the ectoderm and mesoderm in the determination of the forelimb, opinions are very divergent. On the one hand, according to HARRISON (1918) and BALINSKY (1933, 1935 and 1937), all the factors determining the development of the limbs are localized in the mesoderm. On the other hand, FILATOW (1930) first assumed that only the ectoderm contains these factors. Most of FILATOW's experiments were, however, made at a late tail-bud stage, at which the forelimb rudiment was already visible as a thickening of the mesoderm. Later, FILATOW (1932 and 1933) put forward the hypothesis that the general factors determining the position of the limb are localized in the mesoderm. The ectoderm, however, still forms the active part in the development of the forelimb itself.

The heteroplastic transplantations of ROTMANN (1931 and '33) between *Tr. taeniatus* and *Tr. cristatus* showed that the characters of the species concerning the development of the forelimb are localized in the mesoderm. Before the metamorphosis, the ectoderm behaves only passively, but after the metamorphosis an influence of the ectoderm begins to manifest itself, especially in the form of the toes.

Summarising: The ectoderm primarily excites reactive powers to forelimb and somatopleure formation in the whole lateral plate. Then the mesoderm determines the localisation of the forelimb. In the middle stages of the forelimb formation the mesoderm probably forms the most active part, whereas in the further development the ectoderm also influences the differentiation of the limb.

Considering the conception "interaction" as widely as possible, we may say that an interaction between mesoderm and ectoderm occurs in every stage of the forelimb development. In the first phase this interaction has the character of a mutual counteraction of the two layers. In the middle phases the mesoderm forms chiefly the active and the ectoderm the passive part, whereas, finally, in the later phases both are active. So we see that the inductive action emanates more from the ectoderm in one phase and from the mesoderm in another.

My experiments on the development of the lateral plate have elucidated the determination of its medio-lateral axis. The investigations of YAMADA (1937 and '38) are concerned with the determination of the mesoderm along the dorso-ventral axis. The works of BALINSKY (1925-'37), FILATOW (1930-'33) and others relate to the determination of the somatopleure in a cranio-caudal direction.

E. SUMMARY OF PART I.

1) The determination of the outer and inner layers of the lateral plate has been investigated in consequence of the observation of an interesting reduplication of the forelimbs in *Triturus* embryos, in which the whole entoderm had been removed at the early neurula stage.

2) The entoderm was completely removed from neurulae of *Tr. taeniatus* or *alpestris*. Then the ectoderm was folded inwards along the outer border of the mesoderm against the inner side of the chordo-mesodermal plate. In these embryos, the inner layer of the lateral plate, covered by the ectoderm, developed into a somatopleure with all its characteristics.

3) Other entoderm-free neurulae were folded together in a medio-lateral direction, so that the inner layers of the right and left lateral plates fused. They showed a normal development of the outer layers of the lateral plates into somatopleures. In these embryos the fused inner layers, however, formed a very thin partition wall between the two coelomic cavities or hung down in the middle of the fused cavity as an irregular mass of epithelial cells (independent state of development).

4) The ecto-mesodermal envelope of neurulae of *Tr. alpestris* was spread out, and attached itself by its inner side to a thin collodion membrane. It developed into a large disk-like embryo. In such an embryo the outer layer of the lateral plate formed a

normal somatopleure, whereas the inner layer differentiated into a very thin peritoneal epithelium, covering the collodion membrane.

5) The medio-lateral reversal of the presumptive lateral plate mesoderm at the early neurula stage did not influence the development of the larvae. The original outer layer, when covering the entoderm, formed a splanchnopleure. The original inner layer, covered by the ectoderm, developed into a somatopleure.

6) Considering these facts, the following hypothesis is advanced: The ectoderm determines the outer layer of the lateral plate into somatopleure and the entoderm the inner layer into splanchnopleure in the normal development. Without any influence of either the ectoderm or the entoderm an independent development occurs.

7) Supernumerary forelimbs have been formed from the inner layer of the lateral plate, covered by heterotopic ectoderm. Any indication of forelimb formation was absent in the independent state of this layer. These facts prove that the inner layer of the lateral plate has passive limb potencies.

8) Under the primary influence of overlying ectoderm, reactive powers to somatopleure and limb formation and perhaps even differentiation tendencies are activated in the lateral plate layer (increased metabolic activity?).

9) In the following stages the reactive powers and differentiation tendencies to limb formation, at first spread over the whole flank, are restricted to, and concentrated in, the limb areas proper. This is probably caused by a counteraction of mesoderm and ectoderm. On the one hand, the ectoderm now suppresses the limb tendencies while activating the somatopleure tendencies. On the other hand, the mesoderm locally intensifies the limb tendencies and at the same time suppresses the somatopleure tendencies.

10) In the animals, flattened dorso-ventrally, the supernumerary forelimbs originate at the same level as the normal forelimbs. This fact agrees with the supposition that the localization of the forelimb rudiment is brought about by a compound induction from several surrounding organs, which were situated symmetrically in both lateral plates of these animals.

PART II. CHAPTER 1. ORIGIN OF THE GERM CELLS IN THE URODELES.

A. SURVEY OF THE LITERATURE.

Morphological literature.

The origin of the germ cells.

It is not my intention to give a complete survey of the numerous publications dealing with the origin of the germ cells in the various classes and groups of the Invertebrates and Vertebrates. Several authors have already given such a survey, for which reason I may refer to them. I will first mention M. BOUIN, who in 1901 gave a detailed description of articles that had been published previous to 1900. S. KUSCHAKEWITSCH (1910) also gave an exhaustive survey of the literature. Both authors, however, reviewed only the literature on Vertebrates, whereas L. BOUNOURE (1939) has also surveyed the literature on the Invertebrates.

In order to give a general idea of the significance of the morphological literature, I shall merely treat the various problems which have arisen in this field of investigation.

I will restrict myself to the literature on the Vertebrates, as in the Invertebrates other conditions often exert an influence upon the development. The development of the Vertebrates and of most of the Invertebrates differ so much that, in my opinion, the results obtained in one group cannot be extended to the other, except with the greatest caution and reserve. Even in the various classes of the Vertebrates the conditions often vary so much that we have to be extremely prudent in our conclusions.

We shall meet most of the problems connected with the origin of the germ cells and the formation of the gonads, in the Amphibians. I shall, therefore, first discuss the literature on Amphibians, and then extend the discussion to other classes of the Vertebrates also, in so far as new points of view can be gained thereby.

In the last quarter of the 19th century two opposite opinions dominated the literature on the origin of the germ cells; on the one side, the hypothesis of the early segregation of the germ cells from the somatic cells; on the other side, the origin of these cells from already differentiated somatic cells in various periods of the embryonic development and life of the animals. To this question many other questions have been coupled.

The conception of the early segregation of the germ cells is an old one. In the middle of the 19th century OWEN (1849) put

forward such an hypothesis. He studied the parthenogenetic larvae of the Aphides, possessing a group of embryonic cells, which was presumed to form the primordium of the next generation. Other authors, such as GALTON, JÄGER, RAUBER and NUSSBAUM, adopted this view. WEISMANN (1875) proposed a general theory of heredity, based on the idea of the continuity of a "Keimplasma" through the successive generations. We can summarize his view as follows: The fertilized egg contains the immortal "Keimplasma", localized in the nucleus. In every ontogenesis a part of this plasma is reserved for the next generation, and thus its continuity is guaranteed. The moment at which the cytological differentiation of the germ cells begins, differs very much in the various groups of animals; on the one hand, the "Germen" is segregated from the "Soma" in the early development of the embryo; on the other, the "Keimplasma" covers a long way through many generations of somatic cells before it is finally segregated in the germ cells. Not the germ cells themselves are immortal, but only the "Keimplasma" which they contain. In the nuclei of those somatic cells, which are the ancestors of the future germ cells, the "Keimplasma" is still mingled with the nuclear substances of the somatic cells. In the successive cell divisions a separation of the germinal and somatic nuclear substances gradually takes place, after which the germinal character of the cell can express itself.

WEISMANN's hypothesis does not imply the idea of the early segregation of the germ cells from the somatic cells and the cognate idea of the continuous cell lineage of the germ cells, since the "Keimplasma" can as well be localized in somatic cells. In those cases the germ cells are segregated from the somatic cells at later stages of development.

From the investigations on heredity we now know that every cell of an individual has the same genetic properties, for which reason WEISMANN's hypothesis cannot be upheld. The distinction between germ cell and somatic cell must, therefore, be localized in the plasma of the cell.

These considerations have led to the hypothesis of the continuity of the germ cells themselves, and to the theory of an early segregation of these cells from the somatic part of the individual. NUSSBAUM (1880) was the first author who suggested this idea for Vertebrates. The embryonic character of the germ cells – large quantity of yolk granules, amoeboid polymorphic shape of cell

and nucleus, etc. — led him to this hypothesis. In embryonic development the entoderm cells keep their yolk material much longer than the cells of the other germ layers. Therefore he connected the idea of the early segregation of the germ cells with the origin of these cells from the entoderm. On the other hand, according to the same writer, the idea of a mesodermal origin of the germ cells implies a differentiation of these cells from somatic cells.

Over against the conception of the early segregation of the germ cells and the continuous cell lineage of these, WALDEYER (1870) framed his hypothesis of the germinal epithelium. According to his theory, the germ cells originate from the walls of the germ ridges themselves. Thus, the germ cells derive from differentiated somatic cells, *viz.*, the peritoneal cells of the dorsal part of the coelomic cavity. In his opinion no essential difference between germ cells and somatic cells exists. In other words, every somatic cell can be transformed into a germ cell under particular circumstances. In WALDEYER's theory the germ cells can originate only at that developmental stage at which the coelomic cavities have been formed and the germ ridges begin to appear.

These two theories are entirely opposed to each other. Many authors, however, have held opinions bridging the wide gap. A purely peritoneal origin of the germ cells could, namely, not be upheld in consequence of the observation of germ cells at very early developmental stages, long before the beginning of the formation of the germ ridges.

At least 5 different theories on the origin of the germ cells can be distinguished.

1) Early segregation of the primary primordial germ cells. Entodermal origin of all the definitive germ cells of the adult.

In the Anures, many investigators assert that the presumptive germ cells originate from the dorsal entoderm, and separate from this entoderm as a cell ridge. This cell ridge would disjoin as a whole, or in parts, from the entoderm mass.

NUSSBAUM (1880) first recognized the embryonic entodermal character of the germ cells, and assumed their derivation from the blastomeres. He proposed this solely from the external resemblance between both types of cells. The observation of ALLEN

(1907) in *Rana* and of KING (1908) in *Bufo* proved this entodermal origin, which was, moreover, confirmed by WITSCHI (1914) in *Rana* and by many other authors.

I will consider the articles of BOUNOURE (1924-'39) at greater length. With the aid of a special histological staining method, BOUNOURE was able to follow the displacement and fate of the so-called "Cytoplasme germinal" during the development from the fertilized egg up to the adult animal. This "cytoplasme germinal" is situated in the fertilized egg at the vegetative pole, and is displaced during the first cleavages along the cleavage furrows to the centre of the blastocoelic bottom. At the old blastula stage it has partly reached the blastocoel. During gastrulation it is displaced, together with the surrounding cell material, to the ventro-caudal entoderm. Whereas the presumptive germ cells still contain somatic plasma at the early blastula stage, the separation between the somatic and germinal plasmas occurs during the older blastula stage. Shortly before the initial gastrulation the germinal plasma, hitherto lying against the cell surfaces of distinct blastomeres, is displaced in every cell, and finally is found against their nuclei. Then the migration of the germ cells begins. During the gastrulation, they migrate in a ventro-caudal direction. With the commencement of neurulation this direction is altered. They now move to the dorsal entoderm and form the dorso-median germ cell ridge, which is separated from the entoderm. After the formation of the coelomic cavities and the first differentiation of the germ ridges, this median strip of germ cells is divided into two parts. Their cells migrate to the definitive germ ridges. Contrary to the other authors who did not recognize the germ cells before the tail-bud stage, BOUNOURE could trace their whole cell lineage.

According to BOUNOURE a mesodermal origin of the germ cells would mean that these cells arise from somatic cells which already have reached their ultimate destination. On the contrary in the case of an entodermal origin they directly derive from the undifferentiated omnipotent blastomeres.

In the Urodeles, only BOUNOURE (1925) in *Triturus* defends a purely entodermal origin of the definitive germ cells. The germ cells, migrating individually from the entoderm, are displaced dorsad to the median plane, forming a median germ cell ridge; later they migrate to the definitive germ ridges. This investigation, however, begins with stage 25 (HARRISON), which is cer-

tainly too late to allow of studying the first stages of germ cell origin.

2) In deviation from the early entodermal origin of the primordial germ cells, many investigators defend the theory of an early mesodermal origin, especially in the Urodeles. According to them, all the germ cells originate from the mesodermal plates to the left and right sides of the dorsal entoderm.¹⁾

In the Anures only GOETTE (1869) in *Bombinator* and (1875) in *Bufo*, defends a mesodermal origin, without indulging in phylogenetic speculations, whereas CHAMPY (1913) adheres to the gonotome theory.

In the Urodeles, HUMPHREY (1925) argues in favour of a mesodermal origin of the germ cells without, however, excepting the gonotome theory. According to this author, in various Urodeles the germ cells are already recognizable by their light colour at stage 21 (HARRISON) in the most dorsal part of the lateral plate, in the neighbourhood of the Wolffian duct. At stage 25, they are situated close to the entoderm mass.²⁾ They are displaced during the further development to the dorsal median line and then, finally, to the definitive germ ridges. The distinguishing characteristics between germ cells and mesoderm cells increase at older stages, as the latter differentiate rather rapidly, whereas the germ cells hardly change. On the other hand, the marks of distinction from the entoderm decrease during the early development. At the stage at which the germ cells are first recognizable, the entoderm cells are much larger than the germ cells, but during further development the entoderm cells decrease in size by the successive divisions.

In order to bridge the disparity in the origin of the germ cells in Anures and Urodeles – entodermal and mesodermal origin respectively – HUMPHREY has advanced the following hypothesis: In

¹⁾ Many investigators have mingled the interpretation of the facts with phylogenetic speculations. They derived the germ cells from a distinct part of the mesoderm, the so-called "gonotomes". These gonotomes would be situated between the nephrogenous cord (nephrotome) and the dorsal border of the lateral plate. By the presence of such gonotomes they could find correlations between the segmental structure of the gonad of the "most primitive" Vertebrate, *Amphioxus*, and the Selachii and that in the higher classes. (Gonotome theory of RÜCKERT (1888) and VAN WIJHE (1889)).

²⁾ According to HUMPHREY, BOUNOURE has wrongly interpreted this state as the final stage of a migration of the germ cells from the entoderm mass.

both groups the germ cells do not belong to one of the germ layers, but derive from the blastoporal lips of the gastrula, from where they reach the definitive germ ridges by different ways. Moreover, he presumes that "the germ cells may perhaps not be predetermined at early stages (gastrula), but may differentiate either from entoderm or mesoderm under the influence of various environmental conditions".

3) On the other hand, WALDEYER put forward his germinal epithelium theory in 1870. He has many followers. Some of them entirely deny the existence of the primary primordial (p.p.) germ cells (A). The others suggest that all the p.p. germ cells degenerate in the course of the early development of the larva, and are substituted by a new generation of secondary primordial (sec. p.) germ cells, originating from the germinal epithelium (B). A) As for the Amphibians, only one writer, SEMON (1891) in *Ichthyophis*, defends the first opinion, as the p.p. germ cells are here too striking for their existence to be denied. Regarding the other classes of the Vertebrates, we meet this opinion especially with respect to the higher groups, where the germ cells are much more difficult to recognize during the early development. B) Concerning the Amphibians, no single argument in favour of a complete degeneration of the p.p. germ cells has been put forward. With respect to the other groups of the Vertebrates, especially the higher groups, this opinion has found many adherents, however.

Many investigators, who defend an early entodermal or mesodermal origin of the p.p. germ cells, also infer from their observations the appearance of a second generation of sec. p. germ cells from the germinal epithelium ¹⁾. This idea has its origin in two phenomena. 1) The observation of a more or less considerable degeneration of the p.p. germ cells simultaneously with an increase in their number, without the appearance of a sufficient number of mitoses to account for this increase. 2) The observation of many morphological transitional stages between somatic cells and germ cells.

In the "Ueberreifungs-Kulturs" of KUSCHAKEWITSCH the p.p. germ cells were apparently missing under the influence of altered

¹⁾ FIRKET (1914) opines that the p.p. germ cells represent the germ cells of the lower Vertebrates and the sec. p. germ cells those of the higher Vertebrates.

external circumstances. Here they appeared only at older stages. Such a phenomenon forms a strong argument in favour of this opinion.

WITSCHI (1914) criticized this opinion and rejected it on account of various considerations. He, too, observed the degeneration of several p.p. germ cells and the temporary reduction of the germ cell number, followed by a considerable increase. During this increase but few mitoses were to be found. Assuming, however, that a cell division takes about half an hour, here might be as many as 50 divisions a day with but one mitosis present at a time. The greatest increase in the germ cell number observed amounts to about 50 germ cells per day, so that the presence of only a few mitoses at a time suffices for a satisfactory explanation of this increase.

WITSCHI observed the degeneration of a large number of germ cells only in those cultures that had been exposed to unfavourable external conditions. In cultures which had been kept under normal conditions, the germ cells showed no signs of degeneration.

The hypothesis of the transformation of somatic cells into germ cells has been refuted by WITSCHI on the following considerations: 1) After complete castration no regeneration of germ cells occurs. Cf. p. 56-57. 2) The distinction between somatic and germ cells of the germ ridge varies with different staining methods. The oxy- and basochromaticity of somatic and germ cells (used by BERENBERG-GOSSLER) is unreliable, since the germ cells may be alternately oxy- and basochromatic in various periods of their development and division. The stainability of the plasma also changes at the various developmental stages. In this way it is possible that the germ cells are not distinguishable from the somatic cells during distinct periods of their development owing to the temporary disappearance of their characteristics (division periods in birds and mammals). Finally the appearance of basochromatic cells stands in no relation to the strong increase of the number of germ cells. 3) In WITSCHI's cultures at a high temperature transitional stages were entirely lacking. 4) The so-called transitional stages were too rare to prove any transformation of somatic cells into germ cells. 5) In his cultures at low temperatures the notion of a second source of germ cells was entirely superfluous. Degenerating cells did not appear and the number of mitoses was as high as in the cultures with a high temperature.

4) The p.p. germ cells, originating from the entoderm, form a part of the definitive germ cells of the adult; a second generation of germ cells is formed from the germinal epithelium for the above mentioned reasons.

In the Anures, HUMPHREY (1925) does not deny the possibility of the appearance of secondary germ cells. In his opinion the primary source from the medio-dorsal entoderm, however, suffices to explain the number of definitive germ cells on account of the presence of a sufficiently large number of mitoses.

BECCARI (1924) concluded from his quantitative examinations the existence of a secondary germ cell origin from peritoneal cells of the germ ridge, the "paragonia".

Similarly, after BOUIN (1900), the sec. p. germ cells originate after degeneration and extrusion of a rather large number of p.p. germ cells from peritoneal and mesenchymal cells. In his opinion, only the position which a cell occupies determines its final nature.

According to KUSCHAKEWITSCH (1910) the secondary germ cells derive from the same source; at variance with BOUIN's view, his ♂ embryos, however, showed complete degeneration of the p.p. germ cells. In the ♀ embryos only a small part of the p.p. germ cells formed definitive ova. Like BECCARI, he also came to this conclusion by way of quantitative considerations of the number of germ cells at the successive developmental stages. WITSCHI (1914) criticized his interpretations.

According to GATENBY (1915-'16) the greater part of the germ cells originate from the germinal epithelium, only some p.p. germ cells form definitive germ cells.

In Urodeles, ABRAMOWICZ (1913) argues in favour of an entodermal origin of the p.p. germ cells, which differentiate into definitive germ cells. On account of the degeneration and simultaneous increase of the number of germ cells, however, she also comes to the conclusion that a second generation of germ cells from the germinal epithelium must be present. Her investigation begins, as does BOUNOURE's, at a rather late stage (late tail-bud stage).

5) Most of the investigators, who defend an early mesodermal origin of the p.p. germ cells, also admit a second generation of germ cells from the germinal epithelium. On the one side, the degeneration and simultaneous increase of the germ cell number and on the other, the

presence of transitional stages between somatic cells and germ cells have led to this view. DUSTIN (1908) defends this opinion for the Anures. As to the Urodeles, several authors adhere to the gonotome theory, cf. note on p. 41, but also admit a second generation of germ cells from the germinal epithelium¹⁾.²⁾

The degeneration and extrusion of the germ cells.

Many investigators observed a more or less considerable degeneration of the p.p. germ cells. The majority look upon it as a normal phenomenon. WITSCHI, however, ascribes this degeneration to unfavourable external conditions. Likewise, the extrusion of germ cells into the coelomic cavity during their degeneration occurs, in his opinion, by the same causes.

The migration of the germ cells.

Since the p.p. germ cells have first been observed by many investigators at several places in the embryo, situated at various distances from the definitive germ ridges, an extra-regional origin of the p.p. germ cells must be assumed. Therefore their displacement from the side of origin to the definitive germ ridges occurs in the early development of the embryo before the differentiation of the gonads themselves. This process of displacement has been called the migration of the germ cells.

This migration process occurs in two phases. In the first, the germ cells are displaced dorsad in the direction of the median plane. There they form a median cell ridge, situated dorsally to the entoderm mass. In the second, they migrate from this point to the definitive germ ridges. In case of an entodermal origin the first phase is again divided into two stages, viz., 1) the displacement of the germ cells within the entoderm and 2) their separation from this layer and migration to the median germ cell

¹⁾ Regarding the Anures and the Urodeles, DUSTIN has introduced many phylogenetic speculations in the interpretation of the facts. Concerning the origin of the germ cells he holds the following opinion: The primitive Vertebrates, for instance *Petromyzon*, contain only p.p. germ cells. In the Amphibians the p.p. germ cells degenerate for the greater part, and are substituted by secondary germ cells from the germinal epithelium. Finally, in the Amniotes, p.p. germ cells are lacking, and only sec. p. germ cells are formed.

²⁾ KUSCHAKEWITSCH (1910) assumes that the germ cells originate from the entoderm in Invertebrates, whereas the mesodermal origin is characteristic of the higher Vertebrates. The Amphibians represent an intermediate state.

ridge. A similar division can be made in the case of a mesodermal origin, viz., 1) the migration of the germ cells to the Wolffian ducts and 2) the displacement from these areas to the median germ cell ridge.

Concerning the migration process three different opinions are defended by authors above-mentioned.

1) **Active migration of the germ cells.** According to many writers, the germ cells have an amoeboid motility. The polymorphous contour of cell and nucleus form an argument in favour of the present hypothesis. Moreover, the displacement of the germ cells at late developmental stages points unmistakably in this direction. In these embryos, the coelomic cavities have already been formed, so that the cells must migrate to the definitive germ ridges along the dorsal mesentery.

In the Anures, in which the germ cells probably originate from the entoderm, many authors uphold an active migration of these cells from their place of origin to the definitive germ ridges. ALLEN (1906) and BOUNOURE (1937-'39) observed indications of an amoeboid motility of the germ cells.

In the Urodeles, ABRAMOWICZ (1913) and BOUNOURE (1925) assume an individual migration of the germ cells from the entoderm to the lateral plates, and then to the dorsal median line. SPEHL and POLUS (1912) in *Ambystoma* assume an attractive action exercised upon the germ cells by the definitive germ ridges.

2) **Displacements of the germ cells partly by an active migration and partly by a passive transport** by means of growth processes in the surrounding tissues.

PERLE (1927) in *Bufo*, and CHENG (1932) in *Rana* assume an active migration of the germ cells through the entoderm mass. According to the last-mentioned authors, the separation of the germ cell ridge, as a whole, from the dorsal entoderm, points to a passive displacement. This would occur by growth processes of the dorsal borders of the lateral plates. The migration of the germ cells from the median germ cell ridge to the definitive germ ridges may again be an active one.

OKKELBERG (1921) in *Entosphenus* (Fishes) opines that the first displacements within the entoderm of the large germ cells rich in yolk, is a passive one. They probably migrate, actively, however, after their yolk material is consumed.

In birds, SWIFT (1914), and DANTSCHAKOFF (1908, '31 and '32)

in chicken, assume an amoeboid motility of the p.p. germ cells, whereby they migrate into the mesoderm and the blood-vessels. The transport of the germ cells from their extra-embryonic position to the germ ridges, however, occurs passively by the blood current. The migration from the blood-vessels to the germ ridges must be active again.

3) Finally, HUMPHREY (1925) in Anures and Urodeles, and ASAYAMA (1940) in *Triturus*, defend the view of an entirely passive transport of the germ cells by growth processes of the surrounding tissues.

Extra-regional germ cells.

In connection with the place of origin and the mode of migration of the germ cells, the heterotopic positions of the extra-regional germ cells have been explained in different ways. Most of the authors consider them as germ cells which have remained behind on their normal way of migration from the place of origin to the definitive germ ridges. More peculiar positions are explained by the assumption of abnormal ways of migration of the germ cells. According to HUMPHREY, their positions have been caused, however, by abnormal relations in the growth processes. Other authors, who adhere to the germinal epithelium theory or at least admit a second generation of germ cells from the germinal epithelium, mesenchyme or other tissues, give another explanation. They take it that these extra-regional germ cells may also represent cases of extra-genital differentiation of germ cells from somatic cells. (GATENBY (1916)).

Almost every investigator assumes that these extra-regional germ cells degenerate in the course of development; only in extremely rare cases may they give rise to the formation of heterotopic gonads. WITSCHI (1914) and CHENG (1932) in *Rana*.

The development of the germ ridges.

I will restrict myself to the first developmental stages of the gonad formation.

In the publications on the early development of the gonads, one, two or three different successive forms of gonads are distinguished; viz., 1) The bilateral metameric gonotomes. 2) The median germ cell ridge. 3) The definitive germ ridges, which differentiate into ovaries and testes. The first two exist only for a short period in the early development of the embryo.

The opinions regarding the significance of 1) and 2) vary greatly¹⁾, whereas every author admits the existence of the definitive germ ridges situated on both sides of the dorsal mesentery. They are formed as longitudinal folds of the dorsal peritoneum of the somatopleure, and first consist of a thickened peritoneal layer, filled up with germ cells and probably with some mesenchyme cells. In the further development, the germ ridge grows out to a fold which, in cross section, is pear-shaped. After the formation of the mesonephros tubules, rete cords grow out to the germ ridges and penetrate into the mesenchyme, occupying the centre of the fold (primary gonad cavity). In this way, the indifferent gonad is formed.

Most authors, such as DUSTIN (1908) and SCHAPITZ (1912), defend a peritoneal origin of the follicle cells. The mesenchyme cells of the gonad in their opinion derive from the axial mesenchyme. Authors, such as NUSSBAUM (1880), HOFMANN (1886, '89 and '92) and GEMMILL (1896), however, have assumed that the follicle cells originate from dedifferentiated germ cells. ABRAMOWICZ (1913) denied the incorporation of cells from the axial mesenchyme into the gonad. She opines that all the somatic cells of the gonad have a peritoneal origin.

In the 19th century the writers still held various opinions concerning the origin of the rete cords. The later investigations of WITSCHI (1914), SWINGLE (1921), MAC-COSH (1930), CHENG (1932), and others, however, have proved that these cords originate from the mesonephros blastemes.

Finally, I have to deal with the question of the relation between the formation of the germ ridges (definitive gonads) and the presence of the p.p. germ cells. Regarding this question divergent

¹⁾ The adherents of the gonotome theory take the bilateral metamerie gonotomes to be the most primitive gonads of the Vertebrates (cf. the gonads of *Amphioxus*). They consider the median germ cell ridge as the subsequent state of development of the gonad (cf. the median gonad of the *Petromyzontes*). Finally, the definitive germ ridges would represent the gonads of the higher Vertebrates. In Amphibia, in their opinion, these three stages of the gonad-formation occur in the course of development. In the Amniotes, temporary germ cell ridges do not appear during the early development. From this fact DUSTIN (1908) concluded that in the Amniotes only the definitive gonads are formed. Other authors, who do not favour the gonotome theory, deny the existence of the metamerie gonotomes. They look upon the median germ cell ridge only as a temporary accumulation of the p.p. germ cells. Some authors consider the median germ cell ridge as a single strip of cells, whereas, according to others, it represents a bilateral structure.

opinions are again found in the literature. According to KUSCHAKEWITSCH (1910), the formation of the germ ridges is influenced by the presence of the germ cells. HUMPHREY (1927-'29) holds the opinion that the p.p. germ cells are indispensable to the normal differentiation but not to the determination and first development of the gonads. BACHMANN (1914) recorded that the germ ridges also developed in sterile parts of the trunk. GATENBY (1923) observed the formation of the germinal epithelium before the immigration of the germ cells. Finally CHENG (1932) described cases of hypogenitalism, in which sterile gonad rudiments had been formed owing to abnormalities in the germ cell formation.

Experimental literature.

Only a few authors have made experimental investigations on this subject. We can divide their experiments into two groups, the experimental embryological investigations, and the experiments made on adult animals. The latter group chiefly contains castration experiments.

Urodeles.

In addition to BOUNOURE's histological investigations SEMBRAT (1934) made a control experiment. He used *Tr. alpestris* and *Ambystoma*. As mentioned in the preceding chapter, BOUNOURE found the presumptive germ cells in the blastula in the centre of the bottom of the blastocoel; in the young gastrula they were situated in the neighbourhood of the blastocoel. At this stage, SEMBRAT extirpated a fairly large group of blastomeres from the bottom of the blastocoel. He operated upon a hundred animals. From his short note, I received the impression that but few animals could be reared long enough. He found a considerable decrease in the number of germ cells in the operated animals in comparison to the controls, and considered this strong evidence of the accuracy of BOUNOURE's observations. Since he does not take into account the great variation in the number of germ cells in normal animals, the examples he gives are hardly conclusive. After a description of my own experiments I shall return to this one.

HUMPHREY has made several experiments to elucidate the origin of the germ cells and the correlation between germ cells and gonads. He (1927) based his experiments upon the assump-

tion that the germ cells derive from the mesoderm, and that they are segregated prior to the formation of the germ ridges.

In his first experiment he removed at stages 32-37 (HARRISON) the Wolffian duct, the ventral border of the myotomes and the dorsal border of the lateral plate on one side of the body. He used *Amb. maculatum* and *jeffersonianum*. In 90% of the cases he observed a complete sterility in the operated area of the young larvae. In some early operated larvae, small germ ridges or gonads were present, but they were always sterile, and chiefly situated at the cranial end of the germ ridge region. In all probability they often represented early developmental stages of fat bodies. In the larvae of *Amb. maculatum*, operated upon at an older stage, only in one case had an ovary-like structure developed. In the series of *Amb. jeffersonianum* small fertile gonads had been formed in 3 of the 9 older larvae.

These experiments indicate that 1) the germ ridges are determined already at the operation stages mentioned above, and that 2) no regeneration of the germ ridges and germ cells takes place after the removal of their presumptive rudiments. Nevertheless two possibilities remain open. 1) The germ ridges and secondary germ cells may be formed only in a particular part of the peritoneum. 2) The development of secondary germ cells may be dependent upon the presence of primary germ cells. In my opinion the experiments do not solve these questions, the operation stages being too old.

In the transplantation experiments of HUMPHREY (1928), operations were performed at stages 21-34 (HARRISON). The grafts contained the ventral borders of the somites, the dorsal border of the lateral plate, and in several cases a part of the Wolffian duct. The early grafts had a remarkably lower capacity for gonad formation than the older ones (older than stage 25 HARRISON). In my opinion this indicates that the determination of the germ ridges, probably, takes place about stage 25 (e.g. from stage 20 to 25). Since all the sterile germ ridges were of very abnormal structure, HUMPHREY concluded from these experiments that in the absence of the primary germ cells no well differentiated gonads can be formed.

In his publication of 1929, HUMPHREY criticized the work of BOUNOURE and ABRAMOWICZ, who defended the theory of an entodermal origin of the germ cells in the Urodeles. The histological investigations of both authors began at stage 25 and older,

whereas HUMPHREY's experiments were partly carried out at earlier stages. His experiments prove that in any case from stage 21 the germ cells are situated in the mesoderm near the Wolffian duct. The localisation of the germ cells at earlier stages, however, remains still obscure.

HUMPHREY's experiments also prove that the germ cells of the right and left sides of the embryo do not intermingle, but remain separated during the whole migration period.

As all his experiments were performed at rather old stages, they do not afford any decisive answer regarding the first origin of the germ cells; some light has merely been thrown upon the problem of the formation of the germ ridges. Especially the time of their determination and their first differentiation, which is probably independent of the presence of the germ cells, has been elucidated to some extent.

ASAYAMA (1940) has another view concerning the time of determination of the germ ridges. He concludes from his experiments that in *Tr. pyrrhogaster* (Boie) the germ ridges are determined at stage 31. He also considers the intermediate mesoderm not only as a source of germ cells, but also as the pre-primordium of gonad, fat body and mesonephros.

Anures.

The majority of authors hold the opinion that in the Anures the germ cells are separated from the dorsal entoderm at a rather late stage in the form of a median germ cell ridge.

According to BOUNOURE, the so-called "cytoplasme germinal" is situated in the uncleaved egg at the vegetative pole. In 1935 and '37 he irradiated the vegetative pole of the fertilized egg with ultra violet rays for 1 minute. The larvae always contained gonads, but these were atrophic and small. The number of germ cells was much reduced. In all the animals, however, germ cells were still present. In some animals there were but a few germ cells or only one, whereas others contained a larger, but nevertheless reduced, number. According to BOUNOURE, the strong decrease in the number of germ cells has been caused by the destruction of the "cytoplasme germinal". The larvae were examined at the stage of metamorphosis. The reduced gonads showed a fairly normal structure, independently of the number of germ cells. The controls, which had been irradiated at the ani-

mal pole for one minute, showed no reduction either in the number of germ cells or in the somatic part of the gonad. After an irradiation of $2-2\frac{1}{2}$ minutes reduced gonads with decreased germ cell numbers were, however, also observed.

Concerning these experiments, I will first remark that the behaviour of the germ cell material during the development, from the irradiation of the egg till the metamorphosis of the larva (stage of fixation) has not been examined. He has not investigated the direct effects of the irradiation and did not actually observe the destruction of the "cytoplasme germinal". BOUNOURE concluded its occurrence only from the different effects of the irradiation at the animal and the vegetative pole, respectively. These effects do not differ essentially, however, for with a stronger irradiation at the animal pole the number of germ cells was also less. Other possibilities, therefore, remain. For instance 1) The formation of the p.p. germ cells has not been disturbed. Only the development of the somatic tissues of the animal has been influenced by the ultra violet rays. Therefore the germ cells have for the greater part degenerated under the unfavourable internal conditions caused by the irradiation. This seems the more probable since the experimental animals actually show reduced gonads, and since, with a longer period of irradiation, the mortality in BOUNOURE's experiments greatly increased. 2) Only the division of the germ cells may have been disturbed by the irradiation, with the result that the germ cell number has not been increased during development. 3) The irradiation has affected only the inductive centre, whereby the determination of the germ cells has been interfered with. All these possibilities have not yet been examined. Especially the development of only one, or a few, germ cells gives rise to the above criticism. It seems improbable that after the irradiation only so small a part of the "cytoplasme germinal" remains intact that no more than one germ cell can be formed.

In my opinion, BOUNOURE's experiments prove neither the selective destructive effect of the irradiation with ultra violet rays upon the "cytoplasme germinal", nor the origin of the p.p. germ cells from the vegetative plasma of the egg. The results merely point in that direction.

MONROY (1939) removed the whole, the dorsal, or the ventral part of the entoderm from the caudal halves of early neurulae of *Discoglossus*, and fixed the embryos 5 days after operation.

Embryos, developed from the caudal halves of neurulae with entoderm (series A), contained $\frac{1}{2}$ - $\frac{1}{4}$ of the normal number of germ cells of the controls¹⁾. The removal of the dorsal entoderm (series B) caused no alteration in the number and position of the germ cells. On the contrary, entoderm-free animals (series D), and larvae, from which the ventral part of the entoderm had been removed (series C), were sterile. From these facts, MONROY concluded that the germ cells are localized at the neurula stage in the ventral entoderm. In the course of my investigation, however, another possibility arose: The presumptive germ cells cannot differentiate without a previous determination. For instance, assume that the ventral entoderm contains the induction centre. This acts upon the adjacent mesoderm containing the presumptive germ cells. A mutual displacement of entoderm and mesoderm – caused by the use of a part of the entoderm for the closing of the large wound – may interfere with induction. A smaller number of germ cells may, therefore, be expected in the animals of series A. The facts mentioned above may also be explained by this hypothesis.

These experiments give no decisive answer to the question as to the germ cells originate from the entoderm in Anures. They do actually prove that the entoderm plays an important part in the formation of the germ cells. Together with the numerous histological investigations, however, they render it, indeed, very probable that in the Anures the presumptive germ cells are actually situated in the ventral entoderm at the neurula stage.

HUMPHREY (1927) has briefly recorded experiments performed in order to influence the germ cell migration from the entoderm, *viz.*, by means of a) Chemical influences with LiCl or HgCl₂ a few hours before the beginning of the germ cell migration from

¹⁾ He explained this decrease in number firstly by the general reduction of all organs, in consequence of altered food and mechanical conditions. Secondly he supposed that a part of the entoderm, containing presumptive germ cells, was used to close the large wound. This would bring about conditions preventing the presumptive germ cells contained therein from differentiating or reaching the area of the future germ ridges. As the cells, however, still consumed their yolk material, it is, in my opinion, hardly probable that the food conditions have been altered in these experimental animals in comparison to controls. What is meant by altered mechanical conditions is not clear. I think it possible that shortly after the operation a part of the entoderm – the nutritive yolk material – issued through the large wound, taking a number of enclosed germ cells with it.

the entoderm. b) Temporary reduction of the O_2 supply during the blastula stage. c) Removal of the ventral $2/3$ of the entoderm mass in the caudal half of the body, while the dorsal $1/3$, containing the germ cells, remains intact.

According to HUMPHREY, the migration of the germ cells from the dorsal entoderm to the mesenchyme ventrally to the notochord, would be wholly or partly suppressed by these three methods. After these operations the germ ridges contained very few germ cells or none at all. He further recorded that these germ ridges might develop into sterile or almost sterile gonads, in which secondary germ cells might arise at older stages. These experiments would moreover prove that the formation of the gonad is independent of the presence of the p.p. germ cells.

The results of these experiments are very curious. HUMPHREY did not record the presence of germ cells left behind among the entoderm cells in these embryos. The formation of secondary germ cells in these germ ridges or gonads, however, leads me to doubt whether the p.p. germ cells were really absent. As we shall see, the possibility exists that only the morphological differentiation of the germ cells may have been delayed in these animals.

KUSCHAKEWITSCH (1910) recorded the absence of p.p. germ cells in the gonads of a series of *Rana* larvae, developed from eggs, which remained for several days in the uterus of a "lay-ripe" female (surmaturation cultures). Not till 48 days after fertilization would secondary germ cells have arisen in the developed gonads.

BOUNOURE (1927) contested the exactness of these observations. He examined *Rana* larvae of surmaturation series of 7-12 days in vivo, and of 2-5 days in vitro. He could observe a delayed separation of the germ cells from the entoderm and a delayed formation of the germ ridges only, while the germ cells themselves showed a normal differentiation. In a second surmaturation culture (31 hours) of *Bufo vulgaris* he was only able to establish a larger amount of pigment granules in the germ cells. The latter fact corresponds to KUSCHAKEWITSCH's mention of the presence of cells rich in pigment in the sterile germ ridges. According to BOUNOURE, we have to do here with a retarded, or prevented, differentiation or migration of the germ cells only. WITSCHI (1914) also favours this opinion. He, too, observed the pigmented cells, especially in those parts of the germ ridges where the normal germ cells were absent. He recorded that in the older animals

these pigmented cells evidently changed into typical germ cells. He had, however, no proof of the entodermal origin of these cells.

Conclusion: All these experiments have entirely elucidated the origin of the p.p. germ cells neither in the *Urodeles* nor in the *Anures*. In the *Anures* most of the known facts plead in favour of an entodermal position and origin of the p.p. germ cells, whereas with regard to the *Urodeles* they still give very contradictory views.

Experimental investigations in the other groups of the Vertebrates.

As far as I am aware, no experimental investigations have been made to elucidate the origin of the germ cells in fishes, reptiles and mammals. In birds, on the other hand, extensive experiments have been performed by several authors. I will shortly discuss their results here.

According to SWIFT (1914) the germinal crescent contains the p.p. germ cells before their migration. REAGAN (1916) tried to extirpate the germinal crescent which is situated extra-embryonically between the area pellucida and opaca. Of the numerous embryos operated upon, a few only could be reared to the age of five days. They showed sterile gonads. Similar experiments have been performed by BENOIT (1930) with the aid of ultra-violet radiation, and by DANTSCHAKOFF (1931) by cauterization. They observed the complete absence of p.p. germ cells in the operated animals. Grafts of the sterile gonad-forming area, transplanted to the chorio-allantoic membrane, did not show gonad formation. According to DANTSCHAKOFF, the development of a gonad had been prevented by the absence of the p.p. germ cells. From this fact she presumed that these cells give the impulse to the formation and first differentiation of the gonad. WILLIER (1933) also grafted the gonad-forming area to the chorio-allantoic membrane before the beginning of germ cell migration. In contrast with DANTSCHAKOFF, he actually found gonad formation independently of the presence of the p.p. germ cells. He further recorded that the determination of the gonad proceeds gradually from stages prior to the formation of the germinal epithelium till the stage, at which the genital ridges have been formed. Then the gonad is fully determined. GOLDSMITH (1935) extirpated the same cell material, and obtained the same results. He discussed

the significance of the abnormalities and the early death of the embryos.

DANTSCHAKOFF (1931) extirpated the germinal epithelium area at a stage prior to the beginning of the germ cell migration. In consequence of the malformations caused by the operation, the embryos did not survive the fifth or sixth day of incubation. In these embryos the p.p. germ cells migrated to the splanchnopleure, settled cranially to the normal gonad-forming area, and formed normal gonads. In order to examine the further development of these gonad rudiments, she grafted them upon the chorio-allantoic membrane of another host, and observed the formation of a complete differentiated gonad in an embryo of 15 days. In view of these facts she concluded that the experimental proof of an uninterrupted "Keimbahn" had been given. In 1933, she sterilized the gonad rudiments by Röntgen irradiation. She found a self-differentiation of the gonad, independent of the presence of p.p. germ cells, after the sixth day of incubation.

In contrast to SWIFT (1914), MATSUMOTO (1932) holds the opinion that the germinal crescent is situated in front of the embryonic rudiment. HUKAO (1937) endeavoured to give an experimental decision between these contrary opinions, but his experiments stranded upon technical difficulties.

The experiments on adult Amphibians.

The experiments are restricted to castration and transplantation experiments of the gonad. Whereas in other groups of the Vertebrates gonadectomy has been frequently performed, in the Amphibians but few authors have concerned themselves with this experiment. In several cases, the experiments were made in order to examine the hormonal influence of the gonad upon the secondary sexual characters; only a few authors gave data respecting the possibility of regeneration of ovary and testis after removal of the gonad. I will discuss this question alone.

The first who tried to remove the gonad in an Amphibian was LEVI (1905). He attempted to castrate tadpoles of 8-9 mm of *Bufo* with the aid of a hot needle. He frequently observed a regeneration of the gonads, but he was not entirely certain that the whole gonad had been removed. According to MORGAN and MACNAB (1927) regeneration was absent when both ovaries had been completely extirpated; in case of incomplete castration remaining parts would show hypertrophy. Finally, ADAMS and KIRKWOOD

(1929) recorded a large number of castration experiments. They castrated 72 males and 19 females, and fixed them from 33 days to one year after operation. Only a few animals showed gonadal remains.

We may say that the negative cases indicate that after removal of the whole gonad no regeneration takes place, whereas in the sporadic cases of regenerated gonads castration had not been performed completely.

Castration experiments have also been performed in other classes of Vertebrates, especially in birds and mammals. Here two contradictory opinions are defended; on the one hand, the possibility of a renewed formation of a gonad from the germinal epithelium is admitted; on the other, it is denied. The sex inversion of the right rudimentary gonad after removal of the left ovary complicates the relations in birds.

B. TERMINOLOGY AND ABBREVIATIONS IN CONNECTION WITH MY OWN EXPERIMENTS.

In order to give a more exact localization of the positions of marks in vital-staining experiments of the marginal zone, I have divided the whole marginal zone into 12 parts by distinguishing the following zones. From ventral to dorsal we get, successively, the ventral (15° on both sides of the ventral median line), the latero-ventral (from 15° to 45°), the ventro-lateral (from 45° to 75°), the lateral (from 75° to 105°), the dorso-lateral (from 105° to 135°), the latero-dorsal (from 135° to 175°) and the dorsal (15° on both sides of the dorsal median line) parts of the marginal zone.

In the denomination of parts of the mesoderm, the early rudiment of the germ ridges and the developing gonad there is great unanimity among the various authors. Great diversity, however, can be observed in the terminology of the various stages of the germ cell development.¹⁾ The significance of the various names also differs. It will, therefore, be necessary to define exactly the names used for the various developmental stages of the germ cells, in order to prevent further confusion.

I will use the name "sex cells" to denote all stages of germ cell development together.

¹⁾ OKKELBERG (1921) and BECCARI (1924) have already given short surveys.

The germ cell history may be divided into a number of successive periods. The first period begins with the segregation of the germ cells and ends with the first signs of sex-differentiation in germ cells or gonads.¹⁾ I will term the indifferent germ cells of this first period the "primordial germ cells". Many authors distinguish between primary and secondary germ cells, the first representing the germ cells of the early segregation period and their descendants, the second those from other sources. These latter have developed by transformation of differentiated somatic cells. I did not meet with a single secondary germ cell in my experimental animals, and must even question the existence of these cells. Notwithstanding this, I will use the complete term "primary primordial germ cells" in order to prevent any confusion (abbreviation: (p.p.) germ cells). The term "secondary primordial germ cells" will denote the primordial germ cells derived from differentiated somatic cells as long as the embryos have still an indifferent sexual character.

After the commencement of the sex-differentiation – second period – I will speak of "spermatogonia" and "oogonia". In this publication, however, we shall only meet with the p.p. germ cells.

In the description of the somatic part of the gonad I will use names indicating the origin of the cells; thus, "peritoneal cells" derived from the peritoneum, "mesenchyme cells" from the mesenchyme, "rete cord cells" from the mesonephros blastemes, etc. The term "follicle cells" may include cells of various origin.

In the course of this publication I am forced to introduce and to define some conceptions concerning the regional structure of the trunk. I will distinguish, therefore, the fertile region – that part of the trunk in which germ cells are present – from the germinal region – those segments of the trunk in which germ cells can differentiate. The latter conception is probably connected with the regional induction in the mesoderm along the longitudinal axis of the trunk. In normal animals the fertile region and the

¹⁾ In the early development of gonad and germ cell the sexes are still indistinguishable. In most Amphibians, the first symptoms of the sex differentiation of the gonad and of the germ cells do not coincide; the differentiation of the gonad into an ovary or a testis normally precedes that of the germ cells. Only in Anures, with indirect development of the gonad, may the order of events be reversed.

germinal region almost completely coincide. In experimental animals, which often show a disturbed distribution of the germ cells in the caudal part of the trunk, they may differ very much. In the description of the experimental animals I have used the name "germinal region" also in a general topographical sense to denote that part of the trunk in which, normally, germ cells are present.

List of abbreviations, used in the text figures.

an.f.:	animal field	fl.r.:	forelimb rudiment	pan.:	pancreas
an.p.:	animal pole	g.c.:	germ cell	p.ey.v.:	primary eye vesicle
arch.:	archenteron	g.r.:	germ ridge	p.n.:	pronephros
B.:	blastopore	i.:	intestine	p.t.:	pronephros tubule
b.c.:	blood cell	lat.:	lateral	R.:	right
b.l.:	blood lacune	L.:	left	s.b.:	shoulder-blade
br.:	brain	l.:	liver	s.c.:	spinal cord
caud.:	caudal	l.pl.:	lateral plate	som.:	somatopleure
c.c.:	coelomic cavity	m.:	myotome	spl.:	splanchnopleure
chr.:	chromatophore	mes.:	mesoderm	st.:	stomach
cran.:	cranial	m.pl.:	muscle plate	sup.:	supernumerary
c.t.:	connective tissue	m.n.:	mesonephros	t.b.:	tail-bud
d.:	dorsal	m.z.:	marginal zone	t.ent.:	tail-entoderm
d.f.:	dorsal fin	n.:	notochord	v.:	ventral
ect.:	ectoderm	neph.r.c.:	nephrogenous cord	veg f.:	vegetative field
e.p.:	ect. proctodaeum	n.f.:	neural fold	veg.p.:	vegetative pole
ent.:	entoderm	ns.p.:	nasal pit	W.d.:	Wolffian duct
e.v.:	ear vesicle	n.pl.:	neural plate	y.m.:	yolk membrane
ey.:	eye	n.t.:	neural tube		

C. METHOD OF COUNTING THE P.P. GERM CELLS IN THE GERM RIDGES.

The determination of the real number of germ cells presents great difficulty. In parts of the germ ridges, where there are but two or three germ cells per section, their absolute number can be determined with absolute certainty, albeit with great trouble. For the corresponding halves of the germ cells cut can be found in the adjacent sections, which prevents the germ cells being counted twice. In those parts of the germ ridges however where more than three germ cells are localized in each section, exact counting is no longer possible. As the larger parts of the germ ridges contain more than three germ cells per section, it is evident that the determination of the absolute number of germ cells cannot be performed exactly; therefore this method is of no use here.¹⁾

¹⁾ Moreover, since the number of experimental animals of one series is often considerable, this method is too lengthy and is hardly practicable.

It is for this reason that I have used a relative method in which the determined numbers have but a relative significance. By this method all the germ cells, being cut through the nucleus, are counted in every other section. A dividing nucleus, having a larger diameter than a nucleus in rest, is counted for two units irrespective whether its cell nucleus is met with once or twice in the sections examined. (The germ cells of the right (R) and the left (L) germ ridges and the extra-regional (Ex) germ cells are counted separately.)

I will first consider the question as to how far this method meets with the requirement that the relative numbers determined in this way bear a constant relation to the absolute numbers. The embryos are always sectioned at $8\ \mu$. Nuclei of the germ cells having seldom a smaller diameter than $8\ \mu$, almost every one of them will be cut into two or three parts. One germ cell cannot be counted more than twice, as a nucleus never extends over more than 3 sections ($24\ \mu$). Since in this way a number of germ cells are counted twice, the relative number will be greater than the absolute number. The possibility that germ cells will be overlooked, is very slight, for almost every germ cell nucleus is larger than $8\ \mu$; only a very small one, situated in the centre of a section, might be passed over.

The germ cells lie entirely arbitrarily with regard to the positions of the sections. Moreover, the mean diameter and the variation in the size of the germ cells hardly differ in the separate larvae coming from one female (even of slightly different ages). Therefore we may expect that especially in animals of the same female, the same percentage of the germ cell number will be recorded twice. This method was employed in all my series of experiments, as the relative numbers of germ cells of the experimental animals were always compared to those of the corresponding controls, and the age of both was always the same. Even in the heteroplastic grafting experiments, where the germ cell numbers of the two species were compared with their corresponding controls only, the same method was also used.

The exactness of this method can be checked by counting the number of germ cells in the series of odd and even sections (A and B) of the same animal separately, and comparing the numbers obtained in this way. The values must be equal. The differences observed already cancel each other in large measure by counting together three animals of the same series, cf. M. in

table 1. With a larger number of animals, this source of error may actually be negligible.

TABLE 1.

Number of animal	relative number of germ cells (A)	relative number of germ cells (B)	absolute number of germ cells	proportion between relative and absolute number
<i>Amb. mex.</i> 215/6	114	112	86	1,30 and 1,33
" " 219/20	131	117	85	1,54 " 1,38
" " 235/6	138	138	106	1,30 " 1,30
3 ×	M = 128	M = 122	M = 92	M = 1,39 " 1,32
<i>Amb. mex.</i> 201/2	205	220		
" " 170/1	203	202		
" " 185/6	272	245		
	M = 227	M = 222		

D. EXPERIMENTAL PART. ORIGIN OF THE P.P. GERM CELLS IN THE URODELES.

a) Vital-staining experiments of the entoderm.

Series 4. Displacements of the plasma, situated at the vegetative pole of the fertilized egg.

Description of experiments.

In order to control BOUNOURE's results in the Urodeles, I followed the displacements of the plasma at the vegetative pole of the uncleaved egg of *Amb. mexicanum* and *Tr. taeniatus* by using vital-staining.

The staining experiments were performed by the usual method worked out by VOGT (1925). In order to stain the vegetative pole of an egg, a small cube of agar-agar, impregnated with neutral red or nile-bleu sulfate, was placed at the bottom of a hole made in a layer of wax of an operation dish. The egg, surrounded by its yolk membrane, fits into that hole. The animal pole of the egg looks upwards, while the vegetative pole rests upon the agar-agar. After 20 minutes the vegetative pole of the egg has absorbed the stain sufficiently, and a distinct field is stained darkly. As the egg does not press tightly against the agar-agar, the stained mark is not very sharply outlined. This method, however, suffices for this purpose. (Cf. fig. 12a.)

At various stages the position of the stained plasma was examined by cutting the embryo along the median plane or in cross sections. At early stages, previous to the formation of the tail-bud, the living embryo can be easily cut with a glass needle in any direction desired. Older stages, however, are first fixed by immersion in boiling water for a few seconds. In this way, the tissues become hard and brittle. Such a short immersion does not destroy the vital stain. However, the stain remains no longer adsorbed to the pigment granules and plasma, and begins to diffuse into the surrounding cells. A rapid examination of these embryos is therefore necessary.

Fig. 12 and plate VI give semi-diagrammatic pictures of the displacements of the stained plasma during the development of the

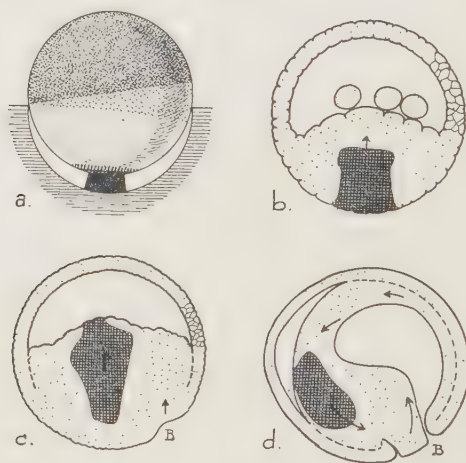


Fig. 12. Vital staining of plasma at vegetative pole of egg in *Amb. mexicanum*. Displacements of stained plasma during cleavage and gastrulation. a) Method of staining. b) Blastula stage. c) Early gastrula stage. d) Yolk plug stage. b-d) Medio-sagittal sections. $\rightarrow$: direction of displacement. Enlargement $\pm 10\times$.

embryo. In fig. 12a the superficial plasma layer of the egg at the vegetative pole has been stained. At the early blastula stage, the stained cells have ascended from the vegetative pole in animal direction to the centre of the vegetative blastomeres (fig. 12b). They have reached the blastocoel at the early gastrula stage (fig. 12c). In fig. 12d, the enlarging gastrocoel has pushed the de-

creasing blastocoel to the ventral side of the embryo, taking the stained cell material with it. By the enlargement of the gastrocoel the stained cell material is displaced still more caudad, so that it is found in the caudal half of the embryo. In the middle neurula (pl. VI, fig. 1a), the stained cells lie medio-ventrally in the caudal entoderm. During the closing of the neural folds and the dorsal edges of the entoderm, the stain-mark retains its position near the anus in the ventro-caudal entoderm (pl. VI, fig. 1b). At the early tail-bud stage, the situation has hardly changed, only the cell material is being stretched in a longitudinal direction. This process continues during the stretching of the embryo. Then the stained cell material forms a longitudinal column, situated in the centre of the ventro-caudal entoderm (pl. VI, fig. 2a). Up to an early larval stage, at which the stretching of the embryo ceases, its position does not change any further (pl. VI, fig. 2b).

Discussion.

The observed ascent of the vegetative plasm to the centre of the egg is in entire agreement with SCHECHTMANN's results (1934 and '35). He had already observed this ascent along the cleavage furrows. At the early blastula stage he found this vegetative plasm in the centre of the vegetative blastomeres, while it reached the blastocoel at the beginning of the gastrulation period.

When the displacements of the stain mark are compared with the displacements of the "cytoplasmic germinal" in the Anures, as described by BOUNOURE, a striking similarity is observed. The only difference is the permanent position of the stained cell material in the ventral entoderm. For in the Anures the presumptive germ cells migrate during the neurulation and the following period of organ formation through the entoderm mass along the dorsal mesentery to the future germ ridges. Similar displacements were not observed in my experiments. For that matter, the displacement of the germ cells occurs in the Urodeles, according to BOUNOURE (1925) and ABRAMOWICZ (1913), by migration of individual cells from the entoderm to the adjacent lateral mesoderm. It could hardly be expected, therefore, that the small stained cells, migrating one by one among the entoderm and mesoderm cells, would be recognizable.

VOGT, SCHECHTMANN, and others, have already made similar vital-staining experiments; my experiments have supplied new

data on the history of the displacements of this entoderm from the fertilized egg till the early larva.

Series 5. Displacements of the superficial cell layers of the ventral half of the vegetative field from the gastrula till the early larva.

Description of experiments.

The displacements of this part of the entoderm have been investigated by VOGT (1925 and '29) and other authors, and the most important data are, therefore, already known. I repeated these experiments merely to obtain a more detailed knowledge of the displacements and positions of this part of the entoderm during the development of the embryo.

The caudal entoderm probably influences the formation of the p.p. germ cells. Its position with regard to the caudal mesoderm, as well as its cranio-caudal extent at the various developmental stages, were, therefore, the subjects of investigation.

As a rule, one or two marks were placed on the entodermal field. In the latter case, a blue and a red mark were used to make a sharp demarcation between them. The blue marks remain visible longer than the red ones.

Of a rather large series of experimental animals, I examined two or three embryos at every stage and, in this way, I was able to reconstruct the whole course of the displacements of this entoderm.

In the eggs of *Tr. alpestris* I stained the ventral half of the vegetative field of a middle gastrula, in which the lateral and ventral

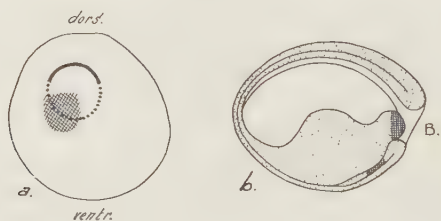


Fig. 13. a) Vital staining of ventral half of vegetative field and lateroventral marginal zone, middle gastrula of *Tr. alpestris* 124. b) Position of mark at slit-blastopore stage, 19 hours, $\pm 20^{\circ}$ C, medio-sagittal section
Enlargement $\pm 10\times$.

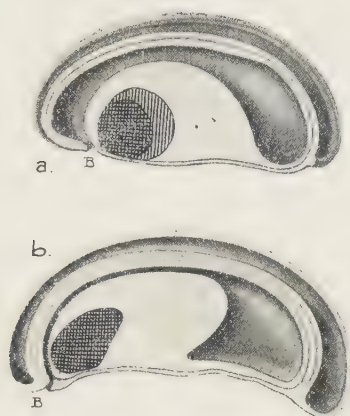


Fig. 1. Displacements of vegetative plasma in *Amb. mexicanum* during neurulation. a) Middle neurula. b) Closed neurula. Medio-sagittal sections. Enlargement $\pm 12\times$.

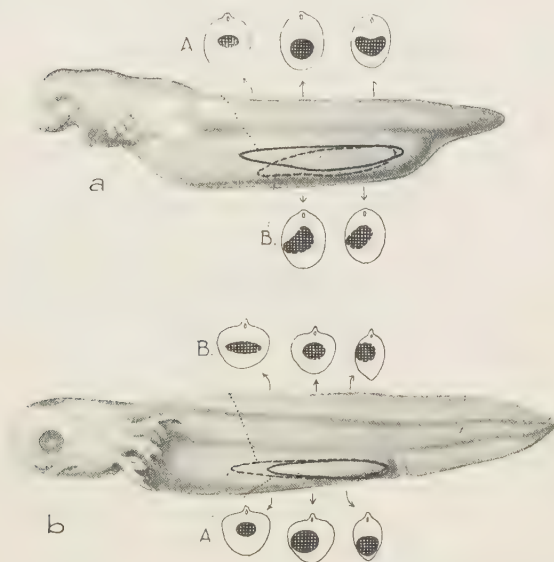


Fig. 2. Displacements of vegetative plasma in *Amb. mexicanum*. a) Tail-bud stage. b) Early larva. Projection of stained plasma upon outside of embryo. Cross sections through entoderm; two observations, continuous line = A, and broken line = B. Enlargement $\pm 8\times$.



borders of the vegetative field were just becoming visible (cf. fig. 13a). After 19 hours, in which the development proceeded to the slit-blastopore stage, the entoderm was entirely invaginated. The invagination of the lateral and ventral mesoderm still goes on. The mesoderm, bordering the ventral half of the vegetative field, has been separated from the adjacent entoderm (yolk plug), and has been invaginated between the caudal ectoderm and entoderm. Fig. 13, in which the blue mark has partly stained the adjacent latero-ventral mesoderm, demonstrates clearly these relations. At the slit-blastopore stage the mark is situated in the most caudal entoderm near the blastopore, whereas the stained mesoderm has been displaced along the latero-ventral side in cranial direction, and has been entirely separated from the stained entoderm.

Fig. 14b shows the lateral extent of the mark, while fig. 14c demonstrates its cranio-caudal extent. The closing of the entodermal walls proceeds from the front backwards; here, the elevation of the entodermal borders has just begun in the caudal part of the embryo (cf. line X-X').

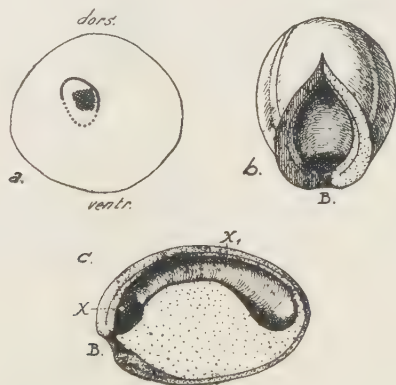


Fig. 14. a) *Id. Tr. alpestris* 19. b) Early neurula, neural plate and archenteron roof cut medially, dorsal view. c) Early neurula, medio-sagittal section, 24 hours, $\pm 20^{\circ}$ C. Line X-X': Lateral edge of entoderm.

Enlargement $\pm 10\times$.

In fig. 15b the blue mark begins exactly at the cranial border of the ectodermal proctodaeum and extends only a short distance in a cranial direction. This figure shows that the stained material has not yet stretched in a cranio-caudal direction. On the con-

trary, the mark has been drawn far out in a medio-lateral direction by the formation of the intestinal tube. Fig. 16b gives a medio-sagittal section; here we can see the cranio-caudal extent of the mark after the closing of the neural tube and the intestinal roof.

At the early tail-bud stage, the ectodermal proctodaeum and the stained caudal entoderm have begun to stretch in a cranio-caudal direction. The stained entoderm occupies twice as large a portion of the intestinal tube as at the late neurula stage (fig. 17b and c). The following day, the embryo has begun to stretch, while the caudal entoderm has already extended a long way. Figs. 18b and c and 19b clearly show the position of the stained entoderm with respect to the neighbouring mesodermal parts. The rudiments of the tail-entoderm can also be seen to the right and left of the ectodermal proctodaeum.

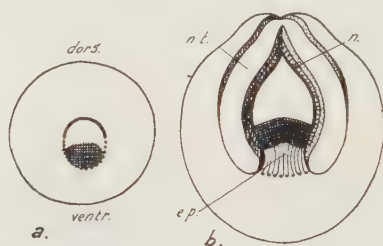


Fig. 15. a) Id. *Tr. alpestris* 254. b) Caudal view, late neurula, cut medio-sagittally through neural tube (n.t.) and archenteron roof (arch. r.), 4 days, $\pm 12^{\circ}$ C. Enlargement a) $\pm 9\times$, b) $\pm 15\times$. E.p.: ectodermal proctodaeum, ...: margin of spread blastopore.

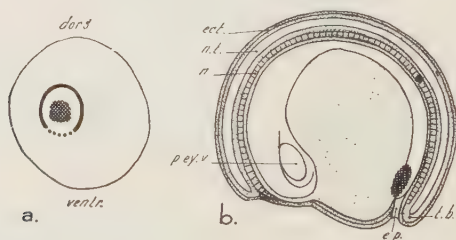


Fig. 16. a) Id. *Tr. alpestris* 129. b) Medio-sagittal section through closed neurula, 4 days, $\pm 12^{\circ}$ C. Enlargement a) $\pm 9\times$, b) $\pm 15\times$. P. ey.v.: primary eye vesicle.

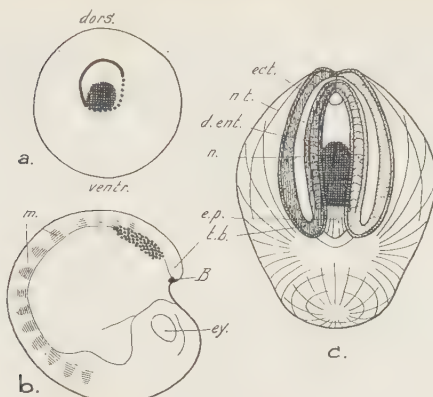


Fig. 17. a) Id. *Tr. alpestris* 141. b) External view at early tail-bud stage, 4 days, $\pm 20^{\circ}$ C. Mark projected upon outside. c) Caudal view, cut medio-sagittally through ectoderm, neural tube, mesoderm and dorsal entoderm; wound margins bent outwards. Enlargement a) $\pm 9\times$, b) $\pm 12\times$, c) $\pm 16\times$. Ey.: eye.

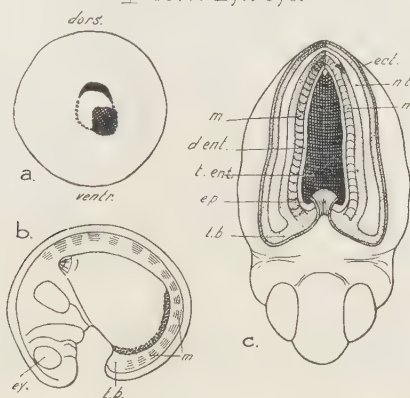


Fig. 18. a) Id. *Tr. alpestris* 63. b) External view at tail-bud stage, 5 days, $\pm 20^{\circ}$ C. Mark stretched, projected upon outside. c) Caudal view, cut medio-sagittally through ectoderm, neural tube, mesoderm, dorsal entoderm and tail bud; wound margins bent outwards. Enlargement a) $\pm 9\times$, b) $\pm 8\times$, c) $\pm 16\times$. T.ent.: tail entoderm.

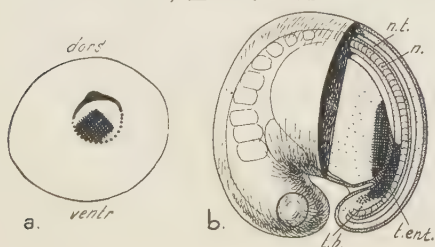


Fig. 19. a) Id. *Tr. alpestris* 163. b) Medio-sagittal section of posterior trunk region, 6 days, $\pm 20^{\circ}$ C. Enlargement a) $\pm 9\times$, b) $\pm 13\times$.

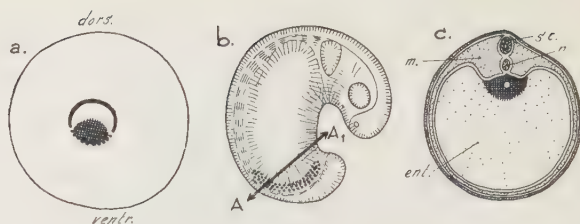


Fig. 20. a) Id. *Tr. alpestris* 41. b) External view, tail-bud stage. Commencement of longitudinal stretching of embryo. Mark projected upon outside, c) Cross section along plane A-A', mark in dorsal part of entoderm, 6 days, $\pm 20^{\circ}$ C. Enlargement a) $\pm 10\times$, b) $\pm 8\times$, c) $\pm 16\times$.

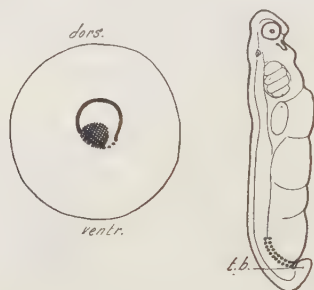


Fig. 21. a) Id. *Tr. alpestris* 74. b) External view of larva. Mark projected upon outside, 7 days, $\pm 20^{\circ}$ C. Enlargement a) $\pm 10\times$, b) $\pm 7\times$.

The position of the stained entoderm has hardly changed in the older larvae (figs. 19-21). Now the embryos are almost completely stretched.

Discussion.

The position of the staining mark at the slit-blastopore stage (fig. 14c) differs from that at the early tail-bud stage, where the ectodermal proctodaeum has been formed (figs. 15b and 16b). It is obvious that the mark has been displaced craniad, since the most caudal entoderm, covered by the ectodermal proctodaeum, has not been stained. In the early neurula the mark borders the ventral and ventro-lateral blastoporal lips and stains the most caudal part of the entoderm. Its position changes, however, during neurulation, and now bounds the cranial border of the ectodermal proctodaeum. The following explanation of this altered position

may be suggested: While the cephalic entoderm extends in a ventro-caudal direction (the embryo as also the gastrocoel forms an almost closed ring), the dorsal entoderm is displaced craniad. In the same way, the ventral entoderm is shifted caudad, the caudal entoderm dorsad, etc. The whole entoderm turns, as it were, round a transverse axis. Hand in hand with this process, the now most caudal entoderm (unstained) is covered by the ectodermal proctodaeum.

During the growth of the tail, and the stretching of the embryo, the whole entoderm is lengthened. Especially the ventro-caudal entoderm is displaced craniad. The tail entoderm grows out from the dorso- and latero-caudal entoderm (figs. 17b and c, 18b and c and 19b).

We can summarize the displacements of the superficial cell layers of the ventral part of the vegetative field – by this staining method only the superficial cell layer can be stained – in the following way: During the last phases of the gastrulation this cell material is invaginated, and forms the bottom of the most caudal portion of the gastrocoel. Its lateral borders are drawn out in a lateral and dorsal direction, and meet in the dorsal median line, forming the intestinal tube. During this process the superficial cell layers are stretched in medio-lateral direction. After the closing of the intestinal tube, the stretching of the caudal entoderm begins. It continues for a considerable time, while the stretching of the embryo begins somewhat later.

As we have seen, the plasma, situated at the vegetative pole of the uncleaved egg, will form other parts of the entoderm than the superficial cell layers of the ventral part of the vegetative field at the successive developmental stages. We have actually to do with two different substances. During further development they remain separated from each other, and form two distinct parts of the entoderm, *viz.*, the ventro-caudal and the dorso-caudal parts. In the course of development the ventral entoderm forms the nutritive yolk syncytium. This is destined to degenerate, and to be digested and absorbed by the intestinal wall (HOLTFRETER (1938)). The dorsal entoderm surrounding the intestinal cavity, develops into the epithelium of the intestine.

b. Transplantation and defect experiments of the entoderm.
Series 6. Removal and transplantation of the vegetative pole plasma of the uncleaved egg at the early gastrula stage.

Experiment and results.

As mentioned above, this material occupies the centre of the blastocoelic bottom at the early gastrula stage. For its removal and transplantation the following method was used: A series of fertilized eggs of *Tr. taeniatum* were stained at the vegetative pole, and then reared for about one day at room temperature till the gastrulation period was reached. After removal of the yolk membrane, the embryos were placed in holes situated two and two in the wax bottom of an operation dish. With a glass needle the roof of the blastocoel was cut over a considerable distance and the wound margins bent over. The stained cell material was removed with two hair loops from the bottom of the blastocoel of the donor (D.) and grafted into the blastocoel of the host (H.). In removing the stained cell material it was unavoidable that overlying and adjacent blastomeres were also extirpated, but this was prevented as far as possible. In this experiment, the early to middle gastrulae are evidently the most suitable, since at these stages the blastocoel is largest, and its roof and walls are fairly firm.

After the transplantation of the stained entoderm, the wound was closed by pressing the wound margins together. As a rule they grew together in a quarter of an hour. The operation must be carried out as quickly as possible, for only fresh wound margins will grow together rapidly. Older ones tend to curl inwards, after which the closing of the wound will be difficult, and anomalies may arise in the roof of the blastocoel, so that the gastrulation may be irregular.

As controls, I made the following series. In the first series, the animals have only been freed from their capsules. In the second the yolk membrane has been removed, the roof of the blastocoel cut, the blastocoel opened, and closed again after a few minutes.

The animals have been fixed at stage 42-43 (GLAESNER) at which the germ cells show hardly any division (first rest-period, which extends over a large number of stages, approx. from stage 35 till stage 44).

Several experimental animals showed abnormalities in consequence of irregularities in the development. The control series showed no abnormalities. In some cases the embryos were considerably deformed in the caudal part of the trunk; they were excluded from the quantitative examination. In the other animals most of the abnormalities were localized in the cranial part of the trunk and in the head, whereas the irregularities in the caudal trunk were negligible.

The results of these experiments follow here.

TABLE 2.

Number of germ cells of un-operated controls (C)		Number of germ cells of operated controls (C')		Number of germ cells of donors (D)		Number of germ cells of hosts (H)	
<i>Tr. taen.</i>	I 499	<i>Tr. taen.</i>	C I 198	<i>Tr. taen.</i>	D I 396	<i>Tr. taen.</i>	H I 281
	II 328		C II 375		D II 322		H II 478
	III 307		C III 537		D III 296		H V 245
	IV 362		C IV 361		D IV 500		H VII 216
	V 373		C V 450		D V 532		H VIII 405
	VI 408		C VI 108		D VI 143		H IX 407
	VII 394		C VII 529		D VII 530		H X 328
	VIII 292		C VIII 271				H XI 388
	IX 320		C IX 125				
	X 209		C X 486				
			C XI 431				
10 × M = 350		11 × M = 352		7 × M = 388		8 × M = 344	
m = ± 24,5		m = ± 43,7		m = ± 55,-		m = ± 32,2	

In every series the average number of germ cells, M , and the probable error of this average, m , have been calculated. The latter has been determined by means of the formula

$$m = \pm \sqrt{\frac{\sum x^2}{n(n-1)}}$$
 where x represents the deviation of each individual value from the average M , and n the number of cases. In comparing two of the averages, M_1 and M_2 , the difference $D = M_1 - M_2$ is supposed to be conclusive, if $D = \text{or} > 3 m_D$,

where the probable error of the difference $m_D = \pm \sqrt{(m_1^2 + m_2^2)}$.

In these experimental series the following values of D , m_D and $\frac{D}{m_D}$ can be calculated. In series I is $D_1 = M_2 - M_1 = 2$, $m_{D_1} = \pm 50,1$

and $\frac{D_1}{m_{D_1}} = 0,04$; in series 2 is $D_2 = M_3 - M_2 = 36$, $m_{D_2} = \pm 70,2$

and $\frac{D_2}{m_{D_2}} = 0,51$ and in series 3 is $D_3 = M_2 - M_4 = 8$, $m_{D_3} = \pm 63,7$

and $\frac{D_3}{m_{D_3}} = 0,13$. The three values $\frac{D}{m_D}$ are all far below the value 3,

so that the differences between the three series have no real significance.

Discussion.

It has to be considered whether we have a right to select our material by omitting a number of highly abnormal experimental animals in the determination of the average of the germ cell numbers. The entirely normal structure of the greater part of the experimental animals demonstrates clearly that the marked abnormalities of some animals may have been caused not by the removal of the blastomeres, but by attendant injuries during the operation and development of these embryos. These injuries which have greatly altered the normal development, have been the causes of the frequently great reduction in the number of germ cells of these animals. This reduction has nothing to do with the experiment itself, and these animals may, therefore, be omitted.

The number of experimental animals of each series is not large. Notwithstanding this these experiments demonstrate with sufficient certainty that in the Urodeles the plasma of the vegetative pole of the egg has not formed germ cells after transplantation into the blastocoel, while, moreover, this plasma is not essential in the formation of the germ cells. ¹⁾ Here we meet the

¹⁾ In 1939 I performed the operation described above with another technique. I used a transplantation pipette, which was inserted between the wound margins into the blastocoel. Then the central blastomeres were sucked up into the pipette and carried to the blastocoel of the partner. This method has the disadvantage among others that it is very difficult to see which parts have been removed. In this series, no. 6', some donors showed a much reduced number of germ cells, whereas others contained a normal or even a very high number.

We may look for the explanation of these conflicting results in the following supposition: In those animals that showed a decreased number of germ cells, not only the blastomeres adjacent to the blastocoel were removed, but also several blastomeres belonging to deeper cell layers. In some cases, the removed cell column actually reached almost to the outside of the gastrula. In the next chapter this discrepancy will be explained. Cf. p. 88 (note).

first great difference between the formation of the germ cells in the Urodeles and in the Anures. In the Urodeles this plasma will probably form only the nutritive yolk material. This set of experiments also demonstrates that a decrease in the quantity of reserve food (removal of the vegetative blastomeres) does not influence the development of the embryo.

Comparing the separate experimental animals, we observe a considerable variability in the individual numbers of germ cells. Values, ranging from 108 to 537, demonstrate the absolute necessity of the use of a large number of experimental animals in every series in order to obtain reliable averages. They further prove that we can conclude nothing from individual experiments. This forms the greatest objection to the conclusions drawn by SEMBRAT (1934) from his defect experiments. He did not take into consideration the normal variability of the individual germ cell numbers, which in the *Triturus* species is very high.

In this series egg material from various females has been used, whereas in the following series the eggs of one or two females only were taken. Comparing the variability in the individual germ cell numbers of both series, it is evident that in the egg material from one female this is much lower than when eggs of various females are used. Compare table 3a, p. 77. In the first case we have to do with the variability of the larvae from one female as well as with the differences between the various females. From the following experiments we shall see that this last mentioned variability is often considerable. Therefore, in the other experiments I have always endeavoured to eliminate this source of error.

Series 7. Removal of the plasma, situated at the vegetative pole of the uncleaved egg.

Experiment and results.

This experiment has been made on *Ambystoma mexicanum*.¹⁾

The fertilized egg, surrounded by its yolk membrane, shows a slit-shaped cavity between the animal half of the egg and the yolk membrane in normal orientation by the gravitation. The

¹⁾ The sharp pigment border between the animal and vegetative halves of the egg is an advantage of the *Ambystoma* egg, whereas the *Triturus* egg has the advantage that the gastrulation is less easily disturbed. The operated embryos of *Ambystoma* often tend markedly to exogastrulation.

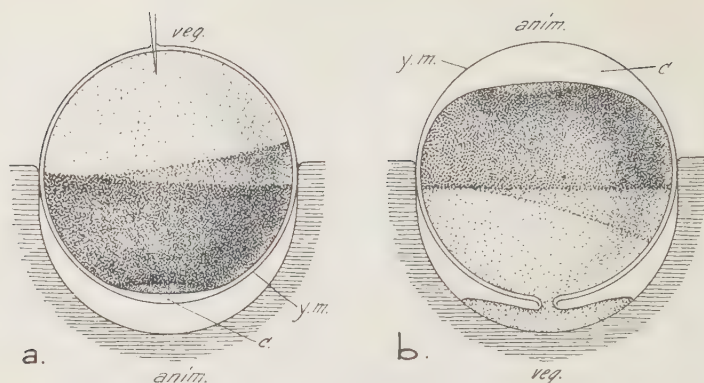


Fig. 22. Operation sketch of removal of vegetative plasma of uncleaved egg in *Amb. mexicanum*. a) Cut through yolk membrane and egg cortex at the vegetative pole. b) Outflow of vegetative plasma. Enlargement $\pm 13\times$. C.: cavity between yolk membrane (y.m.) and egg cortex, an.p.: animal pole, veg.p.: vegetative pole.

yolk membrane is somewhat larger than the egg itself. By the weight of the yolk material – for the greater part localized in the vegetative half of the egg – the egg sinks, as it were, inside the yolk membrane, and orients itself with the animal pole upwards. When the egg is turned with its vegetative pole upwards, it attempts to press the liquid within the yolk membrane up the sides to the upper pole. This displacement of the liquid lasts some time. Just during this time the operation has to be performed.

The fertilized egg, surrounded by its yolk membrane, was turned with its vegetative pole upwards. With a sharp glass needle a cut was made through the yolk membrane and the adjacent egg-cortex exactly at the vegetative pole (the centre of the unpigmented half of the egg). Cf. fig. 22a. By the pressure existing within the yolk membrane (probably osmotic in nature), the contents were pressed out through the opening. The egg-cortex adjoined the yolk membrane round the opening, so that the cavity could not empty. Therefore, part of the egg-plasma itself was pressed out through the wound (fig. 22b). Immediately after the operation the egg was returned to its normal position. The fluid within the yolk membrane absorbing water continuously, the membrane maintained an effective pressure, and the flowing of the plasma went on.

The great difficulty in this operation was to stop this flowing process. If the cut were too large, almost the whole egg was press-

ed out through the opening; if it were too small, the flowing proceeded very slowly and speedily ceased. By perforating the yolk membrane at the animal pole (without injury to the egg), its pressure temporarily dropped to zero, but after some time the flow began again. This process usually went on till the morula stage. Then the osmotic water absorption probably came to a standstill, or the elasticity of the yolk membrane decreased and changed into plasticity.

In this experimental series the operated eggs lost a large content of their vegetative plasma. An exact determination of the quantity of plasma lost cannot be given, but at a rough estimate the egg material was reduced in several cases to 70% of the normal.

Attention has to be paid to a careful orientation of the egg and an exact localization of the cut to prevent a unilateral outflow of plasma, whereby plasma of the marginal zone may also be lost. The boundary between the marginal zone and the vegetative field is recognizable by the different pigmentation of the two areas. Eggs which showed such a unilateral outflow, were, therefore, eliminated.

As might be expected, a fairly large percentage of the operated eggs died in consequence of considerable injuries to the inner structure of the egg, or through loss of the nucleus. In my experimental series 40-60% of the operated eggs died before cleavage began. At a further stage of development other difficulties arose. The operated egg, diminishing in size, with more space within the yolk membrane, gave rise to exo-gastrulation symptoms, which again caused the death of many eggs.

In this series, eggs of two *Ambystoma* females (A and B) were used. Many eggs of female A showed exogastrulation, whereas in the second group, in which the gastrulation proceeded more slowly and equably, but few embryos fell out. In both groups together, 12 larvae were reared up to the age at which their yolk material had been entirely consumed.

In this experimental series, the numbers of germ cells of the left and the right germ ridges, their difference as also the numbers of extra-regional germ cells has been counted. Moreover, the position and relative length of the fertile region in relation to the length of the trunk, have been determined.

The section, intermediate between the two in which the right and the left forelimb rudiments are separated from the body

wall for the first time, has been used as the beginning of the trunk. That section in which the ectodermal proctodaeum communicates with the outer world for the first time, has been taken as the end of the trunk.

In some cases a correction had to be made. In normal animals, the openings of the Wolffian ducts into the proctodaeum lie about 10 sections ($80\ \mu$) behind the end of the trunk (defined above). In some cases, the anal opening

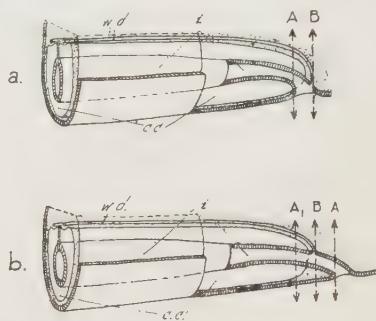


Fig. 23. Diagrammatic sketch of opening of ectodermal proctodaeum (A) and Wolffian duct (B). a) Normal state. b) Abnormal, caudal displacement of anal opening. A': Projected normal opening of proctodaeum. Enlargement $\pm 10\times$.

had, however, been displaced caudad. Instead of a perpendicular bend of the proctodaeum to the ventral side, it ran in a ventro-caudal direction, and ended with a small opening even some sections behind the opening of the Wolffian ducts. In such a case I nevertheless took the section situated about $80\ \mu$ before the endings of the Wolffian ducts as the end of the trunk. Cf. fig. 23b, line A'.

As beginning and end of the fertile region those sections were taken in which the nucleus of the first germ cell of the most cranial, and the nucleus of the last germ cell of the most caudal, group of germ cells were present in the germ ridges. I omitted those solitary germ cells that were situated far in the front or hind part of the trunk, and were separated from the following ones by more than 10 sections ($80\ \mu$). The few extra-regional germ cells, lying outside the fertile region, have also been omitted here.

Table 3a gives the results of this experiment.

The averages of the germ cell numbers of the two control series being nearly equal, general averages of the controls and of the experimental animals have been calculated.

TABLE 3a.

Controls of series A.

Experimental animals of series A.

No: of animal.	Number of germ cells of first germ ridge.	Number of germ cells of second germ ridge.	Number of extra-regional germ cells.	Total number of germ cells.	Difference between germ cell numbers of the two ridges.	No: of animal.	Number of germ cells of first germ ridge.	Number of germ cells of second germ ridge.	Number of extra-regional germ cells.	Total number of germ cells.	Difference between germ cell numbers of the two ridges.
I	179	211	1	391	32	XII	126	127	9	262	1
II	164	172	1	337	8	XIII	130	140	6	276	10
III	120	158	2	282	38	2 ×	M = 269			M = 6	
IV	146	158	5	309	12		m = ± 7,-			m = ± 4,5	
V	160	170	2	332	10		Experimental animals of series B.				
VI	174	176	3	353	2						
VII	151	167	1	319	16						
VIII	176	181	2	359	5	XV	150	166	1	317	16
8 ×	M = 335				M = 15	XVI	84	136	12	234	54
	m = ± 11,3				m = ± 5,-	XVII	68	139		207	71
	Controls of series B.					XVIII	154	209	7	370	55
						XIX	100	116	3	219	16
						XX	91	115	3	209	24
IX	130	171	1	302	41	XXI	100	141	6	247	41
X	151	165	14	330	14	XXII	137	173	13	323	30
XI	183	203	2	386	20	XXIII				377	
M = 339					M = 25	XXIV	269	341	18	628	72)¹)
m = ± 25,-					m = ± 8,2	9 × (10 ×	M = 278			M = 39	
					m = ± 23,-			m = ± 7,-			
					(M = 313			M = 43)			
					m = ± 40,6			m = ± 7,2)			

TABLE 3b.

General average of controls of series A and B.

General average of experimental animals of series A and B.

$M = 336$	$M = 18$	$M = 277$	$M = 32$
$m = \pm 10,2$	$m = \pm 5,9$	$m = \pm 28,6$	$m = \pm 7,1$
		$(M = 306)$	$M = 37)$
		$(m = \pm 35,-$	$m = \pm 6,8)$

¹⁾ In series B one experimental animal showed an extremely large number of germ cells, nearly twice the normal number. The assumption that we had to do with a haploid larva was, however, wrong, as it showed the normal number of chromosomes (about 18). In the first average, this animal is omitted, but in the second (between brackets) it is included.

TABLE 4.
Controls of series A. Experimental animals of series A.
(Averages of right and left sides have always been taken.)

No. of animal.	Number of sections between end of fore-limb and beginning of fertile region.	Number of sections of fertile region.	Number of sections between end of fertile region and beginning of opening of ectodermal proctodaeum.	Total length of trunk.	No. of animal.	Number of sections between end of fore-limb and beginning of fertile region.	Number of sections of fertile region.	Number of sections between end of fertile region and beginning of opening of ectodermal proctodaeum.	Total length of trunk.
I	148	292	40	480	XII	191	220	56	467
II	166	235	65	466	XIII	173	164	69	406
III	171	227	68	466					
IV	160	253	41	454					
V	155	211	95	461	2 ×	± 182	192 ± 28,-	62 ± 5,-	(M)
VI	171	228	67	466					(m)
VIII	139	280	48	467					
Experimental animals of series B.									
7 ×	± 159	249 ± 8,5	61 ± 5,4	465 (M)	XV	187	238	55	480
				(m)	XVI	171	221	42	434
					XVII	242	201	35	478
					XVIII	159	270	40	469
					XIX	193	220	23	436
					XX	214	153	66	433
					XXI	180	167	56	403
					XXII	147	257	46	450
					XXIV	169	258	60	487
3 ×	± 187	239 ± 4,7	42 ± 5,1	467 (M)	9 ×	± 185	221 ± 9,4	47 ± 3,2	(M)
				(m)					(m)

Controls of series B.

The two series have been added and the general averages have been determined.

10 ×	167 ± 33,8%	244 ± 52,4%	55 ± 11,8%	466 (% of total length)	11 ×	184 ± 41,-%	215 ± 47,9%	50 ± 11,1%	449 (% of total length)
				(gen. M)					(gen. M)
				(m)					(m)

Comparing the general average of the experimental animals with that of the controls, the first is somewhat lower than the second. The difference D equals 59. (17,8%) or 30. (9,-%) inclusive of animal XXIV. The disparity between the two sides is, however, on an average, greater in the experimental animals than in the controls. Evidently the difference D is not conclusive, $\frac{D}{m_D}$

being $\frac{59}{30,3} = 2, -$ or $\frac{30}{36,4} = 0,8$ inclusive of animal XXIV.

Table 4 shows the position of the fertile region in the trunk.

The fertile region in the experimental animals is somewhat shorter than in the controls. Notwithstanding the trunk is also shortened, the distances between the forelimbs and the fertile region, and between the fertile region and the anal opening, have somewhat increased.

The difference between the average lengths of the fertile region in the experimental embryos and the controls is, as regards the absolute values, not conclusive; $\frac{D}{m_D} = \frac{27}{15,2} = 1,8$. This difference

is partly due to the diminution in length of the whole trunk. The difference in the procentual value is neither conclusive:

$$\frac{D}{m_D} = \frac{4,5}{3,0} = 1,5.$$

Discussion

The removal of the vegetative plasm at the stage of the un-cleaved egg may have brought about a slight diminution in the number of germ cells, and perhaps also a slight decrease in the absolute, and in the relative, length of the fertile region.

In some experimental animals the quantity of plasma lost during operation, is considerable (more than 30% of the whole cell content). It is, therefore, probable that in these cases not only the plasma of the vegetative pole of the egg, but also some of the surrounding cell material has been lost. This diminution of the presumptive entodermal cell material may have caused the slight decrease in the germ cell number and in the length of the fertile region in some experimental animals (cf. further p. 88 (note)). Several animals, however, show a normal number of germ cells and normal length of the fertile region after a similar decrease in the cell content. The results of this series, therefore, support the

conclusion drawn from series 6. They plead strongly in favour of the explanation that the vegetative plasma of the egg is not essential to the formation of the germ cells. This plasma probably forms merely a part of the nutritive yolk material of the embryo.

Series 8. Removal of the superficial cell layers of the vegetative field in gastrulae.

Experiment and results.

As mentioned above, p. 69, the superficial cell layers of the vegetative field develop into the epithelium of the intestine. The dorsal part of the vegetative field forms the cranial part, *viz.*, the presumptive entodermal part of the pharynx, oesophagus, stomach and duodenum. The ventral part of the vegetative field furnishes the caudal half, *viz.*, the presumptive intestine (s.s.) (Vogt (1929)).

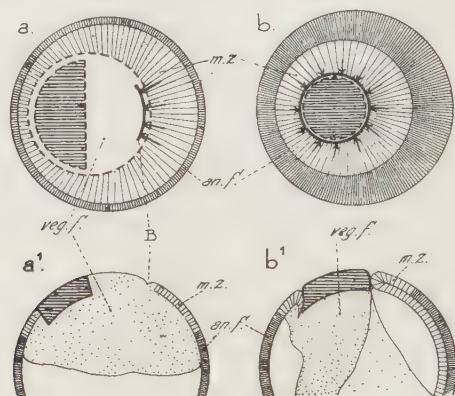


Fig. 24. Diagrammatic operation sketch of removal of superficial cell layers of vegetative field (veg.f.). a) Underside of middle gastrula. Dark hatching: Ventral part of vegetative field to be removed. a') Medio-sagittal section. b) Yolk plug to be removed. b') Medio-sagittal section. Enlargement $\pm 20\times$. An.f.: animal field, m.z.: marginal zone.

In this experiment I employed two groups of gastrulae of *Tr. taeniatus* of different ages. The first group contained early to middle gastrulae with horseshoe-shaped blastopore (stage 8-9, GLAESNER). The second consisted of middle gastrulae (stage 10-11, GLAESNER), in which the outline of the vegetative field had just become visible or a clear-cut yolk plug had already been formed (fig. 24a and b). In the early gastrulae, the ventral half of the vegetative field was removed, while in the older gastrulae the

whole yolk plug was extirpated. The operation was carried out in the two groups in practically the same way.

Eggs without yolk membrane were turned with their vegetative pole upwards. With a sharp glass needle the area to be removed was cut along its borders, after which the superficial cell layers (about the thickness of three cell layers) were extirpated with the aid of two hair loops. The impairment caused by the operation was not very serious. The wound soon closed, probably accelerated by the diminution of the vegetative field during gastrulation. Many larvae developed entirely normally.

In this experiment eggs of two females were used, and therefore two corresponding control series were examined. The larvae were fixed at stage 42-43 (GLAESNER). As there were many extra-regional germ cells in the representatives of this series, only the total number of germ cells was counted. Further, the distribution of the germ cells along the "germinal region" was determined. The whole trunk (from the end of the forelimbs to the beginning of the opening of the proctodaeum) was therefore thought to be divided into nine equal parts, in which the germ cells were counted separately. The length of the fertile region and the number of sections between the forelimbs and the fertile region, and between the fertile region and the anus, were also determined.

The cell material removed in this experiment would have formed the dorso-caudal entoderm at the neurula and older stages. In the experimental animals a defect in this part of the intestine might be expected. The larvae did not show, however,

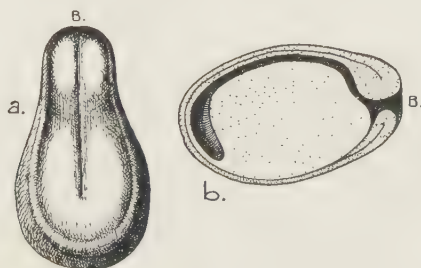


Fig. 25. Control *Tr. alpestris* 133, after removal of yolk membrane, early neurula stage. a) Dorsal external view. b) Medio-sagittal section. Enlargement $\pm 15\times$.

any defect in the intestine, all their parts had been formed entirely normally, so that the defect must have already been remedied. In order to study this regulation, I examined some operated embryos at various stages of their development.

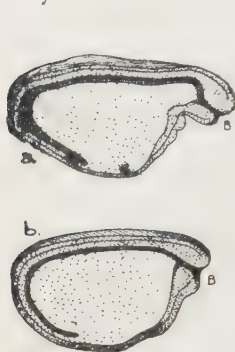


Fig. 26. Experimental animals, *Tr. alpestris* 74 a and b. Removal of presumptive dorso-caudal entoderm at early yolk plug stage. a) and b): Early neurulae, sectioned medio-sagittally. a) with small ventro-caudal defect in ecto- and mesoderm. Enlargement $\pm 14\times$.

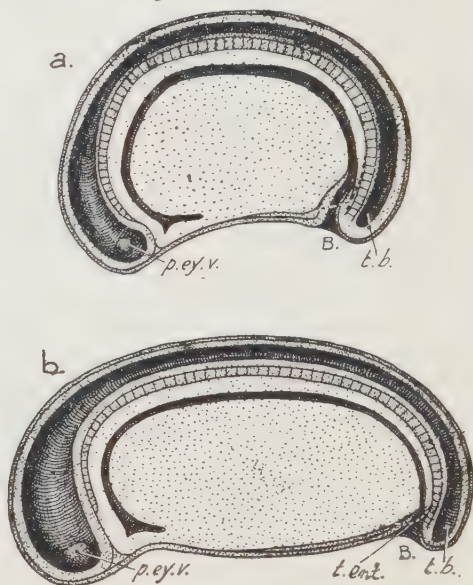


Fig. 27. a) Experimental animal *Tr. alpestris* 174. b) Corresponding control *Tr. alpestris* 124. Early tail-bud stage, sectioned medio-sagittally.

Dotted line: Rudiment of tail entoderm.

Enlargement $\pm 30\times$.

Controls, from which I removed the yolk membrane, acquired an aberrant form during the gastrulation. Instead of the normal round shape, the neurulae now exhibited a pear-shaped form. Cf. MANGOLD (1920). The displacement of the presumptive mesoderm in the direction of the blastopore evidently proceeded more rapidly than its invagination. Therefore, an accumulation of the mesoderm round the blastopore occurred, so that the originally round neurula was changed into a pear-shaped embryo (Cf. fig. 25a and b). The experimental animals showed this deformation in a stronger degree than the controls, probably in consequence of the defect in the caudal entoderm (fig. 26a and b). The experimental and control animals often differed but little, however; the defect in the caudal entoderm had already

been remedied for the greater part. During neurulation, the aberrant form of the embryo disappeared. The accumulated mesoderm had been invaginated and the embryos showed an

TABLE 5a
Controls of series A

No: of animal.	Number of germ cells of first part of trunk.	Number of germ cells of second part of trunk.	Number of germ cells of third part of trunk.	Number of germ cells of fourth part of trunk.	Number of germ cells of fifth part of trunk.	Number of germ cells of sixth part of trunk.	Number of germ cells of seventh part of trunk.	Number of germ cells of eighth part of trunk.	Number of germ cells of ninth part of trunk.	Total number of germ cells.	Number of sections between end of forelimbs and beginning of fertile region.	Number of sections of fertile region.	Number of sections between end of fertile region and beginning of anal opening.	Number of sections of whole trunk.
74			7	44	60	86	65	18	3	283	63	174	30	267
73			3	36	57	58	68	21	6	249	82(92)	188(178)	18	288
72				13	45	62	95	45	6	266	100	158	26	284
71			14	24	55	78	67	14	1	253	58	174	30	262
52			5	18	36	56	43	47	4	209	72	160	22	254
53			3	15	42	63	64	46	23	256	105(87)	182(200)		287
54	2		8	21	61	63	46	9	1	211	69	152	30	251
55			1	42	59	83	48	16	5	254	83	152	28	263
56			4	12	59	70	52	11	4	212	83	172	26	281
57			5	12	45	73	105	62	49	351	86	220	- 2	304
58			11	18	42	74	75	62	35	317	77	204(194)	-10(0)	271
11 X										M = 260 m = $\pm 13,4$	80 29%	176 64%	18 7%	274 100%
Controls of series B														
81			11	39	41	73	37	7	2	210	61	148	24	233
82			7	16	57	83	63	6		232	74	132	50	256
83		2	25	59	93	99	50	20	3	351	50	170	30	250
84			19	25	38	31	33	10	2	158	94	146(132)	24(38)	264
85		20	4	56	50	66	58	7	1	262	79	136	50	265
86			17	36	61	84	72	18	3	291	56	172	22	250
87			11	35	81	107	51	20	2	313	59	160	32	251
88		4	23	34	44	68	45	13	1	232	68	168	24	260
8 X										M = 256 m = $\pm 21,7$ General averages of series A and B = 258 m = $\pm 8,7$	68 27% 28%	155 61% 63%	32 12% 9%	254 100% 100%

TABLE 5b
Experimental animals of series A, group I

No: of animal.	Number of germ cells of first part of trunk.	Number of germ cells of second part of trunk.	Number of germ cells of third part of trunk.	Number of germ cells of fourth part of trunk.	Number of germ cells of fifth part of trunk.	Number of germ cells of sixth part of trunk.	Number of germ cells of seventh part of trunk.	Number of germ cells of eighth part of trunk.	Number of germ cells of ninth part of trunk.	Total number of germ cells.	Number of sections between end of forelimbs and beginning of fertile region.	Number of sections of fertile region.	Number of sections between end of fertile region and beginning of anal opening.	Number of sections of whole trunk.	Class to which each animal belongs on account of the number of its germ cells. Cf. p. 86.
51				21	41	41	21	2		126	94	118	50	262	III
50			4	11	36	73	30	4	2	160	65 ⁽⁸⁵⁾	138 ⁽¹¹⁸⁾	46	249	II
49			1	4	23	13	7			48	117	106	68	291	IV
48					11	19	6			36	128	82	62	272	IV
47			2	23	49	62	53	22	11	222	88	162	10	260	I
42				4	7	18	23		2	54	118	94	60	272	IV
41			5	28	65	75	17	2		192	79	120	66	265	II
40				13	33	16	15	1	1	79	92	100	52	244	IV
39			1	12	26	20	16	4	1	80	90	152 ⁽¹¹⁸⁾	28 ⁽⁶²⁾	270	IV
38				8	35	48	29	4		124	105	120	42	265	III
37			3	26	52	52	31	5		169	80	128	54	262	II

Experimental animals of series B, group I

34				14	26	74	29			143	117	98	38	253	III
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General average of group I, series A and B.

$$M = 119$$

$$m = \pm 17.4$$

Experimental animals of series A, group II

67			8	18	44	52	16	2		140	58	106	40	204	III
46		3	38	34	53	72	22	11	1	234	57	160	38	255	I
45			5	42	63	50	44	10	4	218	82	140 ⁽¹⁵⁸⁾	36 ⁽¹⁸⁾	258	I
44		10	21	54	44	36	7	12	1	185	44	174	22	240	II
43			3	30	43	43	41	15	2	177	76	160	46	282	II
36			1	17	50	68	68	32	2	238	89	140	30	259	I

Experimental animals of series B, group II

35			7	16	43	62	50	10	2	190	75	134	44	253	II
68			10	53	40	35	12			150	57	116	58	231	III

General average of group II, series A and B.

$$M = 192$$

$$m = \pm 12.9$$

General average of group I and II, series A and B.

$$M = 148$$

$$m = \pm 14.7$$

almost normal shape after the closing of the neural folds. At the early tail-bud stage both the experimental and the control animals were of the same structure. The regulation was complete, even the tail-entoderm had been formed normally (fig. 27a and b). They both showed a somewhat elongated shape owing to the absence of the yolk membrane.

From these experiments we may conclude that the defect is probably regulated at the end of the neurulation, or earlier. This has occurred by replacement of the excised entoderm by more ventral and more cranial parts.

Table 5a gives the data regarding the control larvae.

The averages of both series being nearly equal, they have been counted together, and one general average has been calculated.

In the experimental series, I have separated the animals of the two operation stages (group I and II).

Table 5b gives the data obtained from the experimental animals.

The difference between the general averages of the germ cell numbers of all the control and experimental animals amounts to 110 (42,6% of the average number of germ cells of the controls).

As $\frac{D}{m_D} = \frac{110}{18,2} = 6,0$, we may conclude that in this experimental

series a conclusive decrease in the germ cell number has been established. When we determine the difference of the average germ cell numbers of the control and the experimental animals of group I and II separately, we arrive at the following values:

$$D_I = 139 \text{ (53,9\%)}, \frac{D_I}{m_{D_I}} = \frac{139}{20,9} = 6,7 \text{ and } D_{II} = 66 \text{ (25,6\%)},$$

$$\frac{D_{II}}{m_{D_{II}}} = \frac{66}{17,3} = 3,8. \text{ Thus, in both groups the decrease has a real}$$

significance.

The evident difference between the length of the fertile region in the experimental animals and the controls leads to the question of the existence of a possible connection between the number of germ cells and the length and position of the fertile region. In those animals with a considerably lower germ cell number the distribution of the germ cells along the fertile region has evidently not been disturbed, only the length of the fertile region has been reduced. Irregularities in the distribution, e.g., large sterile intervals, do not appear; the distribution of the germ cells shows

a fairly regular curve with a top in the middle of the fertile region. Due to this regular distribution, the germ cells in corresponding parts of the trunk of different animals might be counted together, so that an average curve could be determined. For this purpose I divided the experimental animals into four groups. The first group contained animals with more than 200 germ cells, the second those with 151 up to 200 inclusive, the third with 101 up to 150 inclusive and the fourth with fewer than 101 germ cells. In table 5b, the groups I-IV have been marked in the last column.

Table 6 gives the germ cell distribution and relative length of the fertile region of the four groups and of the controls.

TABLE 6
Experimental animals and controls of series A and B
Average value in each group

No: of group.	Number of germ cells of first part of trunk.	Number of germ cells of second part of trunk.	Number of germ cells of third part of trunk.	Number of germ cells of fourth part of trunk.	Number of germ cells of fifth part of trunk.	Number of germ cells of sixth part of trunk.	Number of germ cells of seventh part of trunk.	Number of germ cells of eighth part of trunk.	Number of germ cells of ninth part of trunk.	Total number of germ cells.	Number of sections between end of forelimbs and beginning of fertile region.	Number of sections of fertile region.	Number of sections between end of fertile region and beginning of anal opening.	Number of sections of whole trunk.	
I	1	11	29	54	63	47	19	5	228	79	151	28	258	(Length and position of fertile region also calculated in % of whole length of trunk.)	
II	2	7	28	47	57	29	8	1	179	31% 70	58% 142	11% 46	100% 258		
III		4	20	35	40	30	8		137	27% 80	55% 112	18% 46	100% 243		
IV			7	20	17	13	1	1	59	35% 109	46% 107	19% 54	100% 270		
C	2	9	29	54	72	60	24	8	258	40% 75	40% 167	20% 24	100% 266		
										28% 63%		9% 100%			

From this table we may conclude that a reduction of the germ cell number is accompanied by a corresponding decrease in length of the fertile region. At the same time the distribution of the germ cells along the fertile region remains unchanged.

Another interesting phenomenon appeared in this experimental series. Parallel with the diminution in the number of germ cells, the histological differentiation of the germ cells also

diminished, by which I understand a reduction in the number of characteristics of the germ cells.

"Well differentiated" germ cells are recognizable by the following characteristics: The nucleus is voluminous (1), and contains one or more homogeneous nucleoli (2). The nucleus has a fine spongy chromatin structure (3). The nuclear membrane is sharply drawn (4). The germ cell nucleus is distinguished from the other nuclei by a weaker stainability (5). The nuclei often have a bilobed or polymorphous shape, and are seldom rounded (6). The most typical characteristics are to be found in the cell plasma. The nucleus is surrounded by a broad zone of hyalin plasma (7), bordered by a distinct cell membrane (8). In the plasma, many scattered brown pigment granules are visible, sometimes concentrated into large clouds (9).

In "Badly differentiated" germ cells, on the contrary, many of these characteristics are lacking: The nucleus is smaller; parallel with this reduction in size, the stainability of the chromatin often increases (concentration of the nuclear substances?). Although the characteristics of the nucleus may disappear for the greater part (except the large round nucleoli (2)), the characteristics of the plasma remain visible. Even in spite of a greatly reduced size of the cell, a distinct plasma zone (7) with scattered pigment granules (9) can always be observed.

Discussion.

Why do the operated gastrulae of group I show a greater decrease in the germ cell number than the embryos operated upon in the yolk plug stage? The extirpated quantities of entoderm cells have probably been larger in the first group than in the second. During the gastrulation the whole vegetative field decreases in size, and is gradually invaginated from dorsal to ventral. In the older gastrulae a large part of the superficial, but especially of the deeper, cell layers (pushed away in a cranial direction) have already been invaginated and therefore escape removal. We may therefore assume that the removed yolk plug has been smaller than the ventral part of the vegetative field of the early gastrula.

In the preceding experiments a large quantity of entodermal blastomeres, or nutritive yolk material, has been removed without any decrease in the number of germ cells. The question

as to whether the reduction in the germ cell number might be caused by a decrease in the quantity of reserve food, may, therefore, be definitely answered in the negative.

Surveying the results of this experimental series, we may conclude that the dorso-caudal entoderm, in contradiction to the ventro-caudal entoderm, has an important rôle in the formation and differentiation of the p.p. germ cells¹⁾. Cf. further p. 111-114.

C. Vital staining experiments of the mesoderm.

Series 9. Vital staining experiments of the caudal mesoderm.

Description of experiments.

VOGT (1926 and '29), YAMADA (1937 and '38), BIJTEL (1929-'31), NAKAMURA (1938), and others have already made exhaustive investigations respecting the displacements of the cell material of the marginal zone. Nevertheless, the data obtained concerning the displacements of the presumptive lateral plate mesoderm were still incomplete, especially in quantitative respect. I repeated these experiments for the purpose of amplifying the experimental data.

My experiments on extirpation and heteroplastic transplantation of the presumptive lateral plate and nephrogenous cord mesoderm (cf. below, p. 102 and 129) have been made at the early neurula and yolk plug stages, respectively. I therefore chose the yolk plug stage as starting point for my vital staining experiments. All these experiments of vital staining have been performed on eggs of *Tr. alpestris*, and were carried out in the usual manner.

Description of the development of the embryos with vital staining.

Tr. alpestris 165 (fig. 28). At the yolk plug stage, two marks (a red and a blue one) were applied to the material on either side of the yolk plug at some distance from the blastoporal lip. After 21 hours, they had been partly invaginated. The positions of the external parts of the marks had not been altered,

¹⁾ The slight reduction in the number of germ cells and in the length of the fertile region in series 6' and 7 may have been caused by the removal of a part of the dorso-caudal entoderm too. In series 6' deeper cell layers have also been extirpated in some experimental animals. These deeper layers already belong to the superficial cell layers of the vegetative field. In series 7, plasma, adjacent to that of the vegetative pole, has, perhaps, also flowed out in some cases through the wound in the egg cortex. This assumption may explain the more or less aberrant results of these experiments.

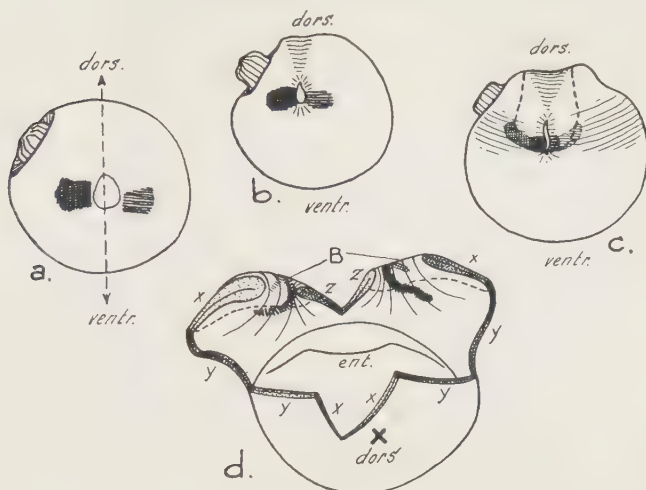


Fig. 28. Vital staining of lateral blastoporal lips, *Tr. alpestris* 165. a) Application of two marks at middle yolk plug stage, caudal view. b) External position of marks at slit-blastopore stage, caudal view, 21 hours. c) External and internal (fine) position of marks at early neurula stage, caudal view, 25 hours. d) Ecto-mesodermal envelope of neurula, opened by successive cuts: X: Dorso-median cut, Y: Transverse cut, Z: Ventro-median cut, dorsal view, 25 hours. Continuous lines: Meridians, broken line: Original caudal border of entoderm. Enlargement 10-12 $\times$.

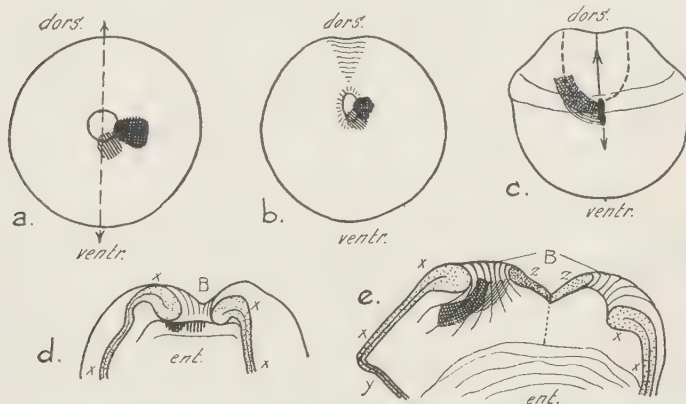


Fig. 29. Staining of lateral and latero-ventral blastoporal lips, *Tr. alpestris* 154. a) Application of marks at middle yolk plug stage, caudal view. b) External position of marks at slit-blastopore stage, caudal view, 21 hours. c) Internal position of marks at early neurula stage, caudal view, 24 $\frac{1}{2}$ hours. Dotted line: Dorsal border of entoderm. d) Ecto-mesodermal envelope opened, early neurula, dorsal view. e) Id. without entoderm, dorsal view, 24 $\frac{1}{2}$ hours. Cuts cf. fig. 28. Continuous lines: Meridians. Enlargement $\pm 12\times$.

they still stained the lateral blastoporal lips. After 25 hours, at the early neurula stage, the positions of the marks were determined by cutting the ecto-mesodermal envelope.

The first remarkable fact is the great size of the marks in a cranio-caudal direction. At the yolk plug stage the marks were almost square in shape. At the early neurula stage they formed long coloured bands, lying partly at the outside, but chiefly on the inner side of the ecto-mesodermal envelope. The position of the invaginated parts of the marks forms a second interesting fact. As soon as the cell material passed the blastoporal lip and reached the entoderm (--- line), it bent sharply dorsad. Comparing the positions of the invaginated parts of the marks with the meridians drawn on the sketch at the inner side of the opened ecto-mesodermal envelope, the dorsal deviation of the mark becomes very clear.

Tr. alpestris 154 (fig. 29) presents the same aspect. Here, moreover, a ventro-lateral staining mark was applied. At the early neurula stage this mark had also been displaced to the dorsal side, and occupied the inner lateral side of the mesodermal envelope.

Tr. alpestris 180 (fig. 30). The two marks had a medio-ventral position on the blastoporal lip. At the early slit-blastopore stage, the invaginated marks stained the medio-ventral mesoderm. The medio-ventral mark had been considerably drawn out in a lateral direction, and was now triangular in shape. Here the dorsal convergence movement could already be observed, but it had not reached such a degree as at the neurula stage. (Compare *Tr. alpestris* 154 and 156).

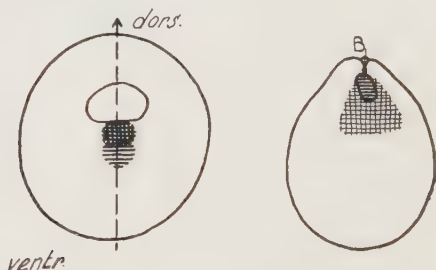


Fig. 30. Staining of medio-ventral blastoporal lip, *Tr. alpestris* 189. a) Application of marks at yolk plug stage, ventro-caudal view. b) External (dark) and internal (fine) position of marks at slit-blastopore stage, ventral view, $17\frac{2}{3}$ hours. Enlargement $12-14\times$.

Tr. alpestris 170 (fig. 31). Here two marks (a blue and a red one) were applied to the ventral blastoporal lip at the early yolk plug stage. They were situated almost entirely to the left of the median plane. After $17\frac{1}{2}$ hours the blue mark had been wholly invaginated and the red one, probably, in part. The blastoporal lip being rather thick, the invaginated part of the red mark was not to be seen externally. The invaginated blue mark had a latero-ventral position. At the slit-blastopore stage the movements of dorsal convergence had not yet fully begun. During the closing of the neural tube and the following formation of head and tail-bud, the greatest displacements

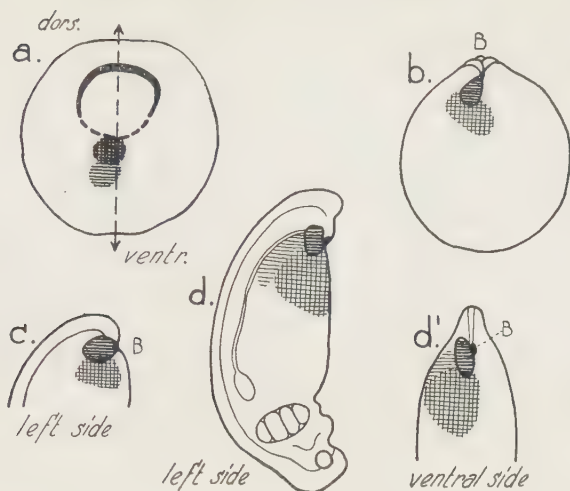


Fig. 31. Staining of ventral blastoporal lip, *Tr. alpestris* 170. a) Application of marks at early yolk plug stage, ventro-caudal view. b) External (dark) and internal (fine) position of marks at slit-blastopore stage, ventro-caudal view, $17\frac{1}{2}$ hours. c) Id., early tail-bud stage, left lateral view, $41\frac{1}{2}$ hours. d) Id., later tail-bud stage, left lateral and ventral views, 66 hours. Enlargement a-b $\pm 12\times$, c-d $\pm 7\times$.

took place, especially in the medio-ventral and latero-ventral mesoderm.

After $2\frac{3}{4}$ days, the embryo reached the tail-bud stage. The blue mark now occupied a large field in the ventral to lateral mesoderm. The whole caudal part of the left lateral plate, and the caudal part of the presumptive blood-island, had been stained by it. The successive displacements of the cell material during the intervening stages could not be traced in this case; only the final state was studied.

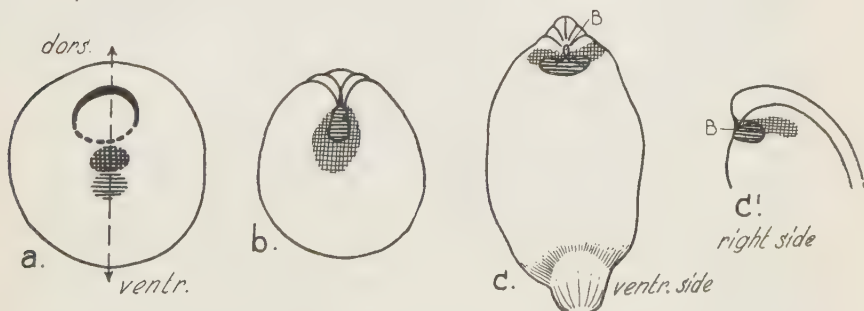


Fig. 32. Staining of medio-ventral blastoporal lip, *Tr. alpestris* 188. a) Application of marks at early yolk plug stage, ventro-caudal view. b) External (dark) and internal (fine) position of marks at slit-blastopore stage, ventro-caudal view, $17\frac{3}{4}$ hours. c) Id., early tail-bud stage, ventral and right lateral views, $41\frac{1}{4}$ hours. Enlargement $\pm 12\times$.

This animal demonstrates a further interesting fact. The red mark, situated farther from the blastopore, had also been partly invaginated. Instead of forming the most caudal part of the medio-ventral and latero-ventral mesoderm, it stained the dorso-lateral mesoderm.

Tr. alpestris 188 (fig. 32) showed a blue and a red mark, applied to the external ventral blastoporal lip near the median plane. The blue mark was situated at some distance from the ventral border of the vegetative field, and thus did not stain the first invaginating mesoderm. At the early slit-blastopore stage, the blue mark had been entirely invaginated and showed a rounded form. The next day its form had considerably changed. It was now bilobed, the middle lines of the two lobes making an angle of about 180° .

Tr. alpestris 156 (fig. 33) clearly demonstrates the displacements of the medio-ventral mesoderm at the successive developmental stages. Here, two marks were applied in a medio-ventral position; the first, a blue mark,

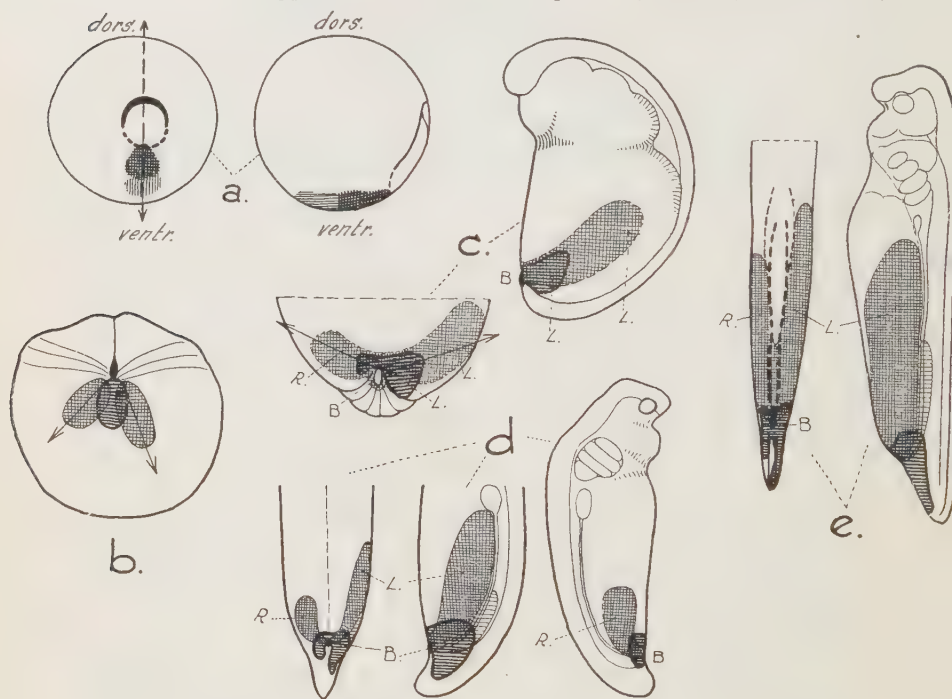


Fig. 33. Staining of medio-ventral blastoporal lip, *Tr. alpestris* 156. a) Application of marks at early yolk plug stage, caudal and right lateral view. b) External (dark) and internal (fine) position of marks at early neurula stage, caudal view, $19\frac{1}{4}$ hours. c) Id., eye vesicle stage, ventral and left lateral views, $44\frac{1}{2}$ hours. d) Id., tail-bud stage, ventral, left and right lateral views, $67\frac{3}{4}$ hours. e) Id., early larva, ventral and left lateral views, 91 hours.

Enlargement a-c) $\pm 12\times$, d-e) $\pm 9\times$.

bordered the ventral side of the vegetative field; the second, a red one, had, a more animal position on the external marginal zone.

At the early neurula stage, the blue mark had been entirely invaginated, and was bilobed in form (shimmering through the ectoderm). Its medio-ventral part was already very small. The middle lines of the right and left lobes made an angle of about 70° with each other.

At the early tail-bud stage, this angle had increased to $\pm 145^\circ$. The next day, $67\frac{3}{4}$ hours after the staining of the ventral blastoporal lip, the stretching of the embryo had commenced. Now the mark extended still further in a cranio-caudal direction. On the left side it reached to the caudal end of the pronephros rudiment. Besides this longitudinal stretching, the blue mark had also extended in a dorso-ventral direction. Dorsally it now reached up to the Wolffian duct and the ventral border of the myotomes, and in ventral direction it covered $\frac{2}{3}$ of the caudal entoderm mass. The angle between the two lobes had decreased, and the interjacent field formed again a narrow band. During the next 24 hours this process continued. In cranio-caudal direction, the mark still extended for some length, but especially in ventral direction it broadened considerably. The two lobes now surrounded almost the whole entoderm, only a very narrow median strip of mesoderm remained uncoloured. At this developmental stage we can distinguish the various parts of the mesoderm (myotomes, nephrogenous cord, lateral plate and blood island). It was now clear that the blue mark had stained the lateral plates entirely (in dorso-ventral direction) on the right and left flank, while the interjacent medio-ventral strip represented the blood island, the most caudal part of which, however, had probably been also stained by the mark. The figures 32 and 33 show that part of the red mark had been invaginated too. It stained the caudal part of the left nephrogenous cord. (The red mark lay asymmetrically in respect to the ventral median line).

In this animal the blue mark stains the whole lateral plate in dorso-ventral direction in the early larva, and thus we can project the animal-vegetative extent of the presumptive lateral plate mesoderm at the early yolk plug stage. It occupies an area extending 30° in animal direction from the ventral border of the vegetative field. Cf. fig. 35a. *Tr. alpestris* 171 yields the same value.

Tr. alpestris 173 (fig. 34) gives an example of the determination of the dorso-ventral extent of the presumptive lateral plate and nephrogenous cord mesoderm at the early yolk plug stage. At this stage, three marks had been applied, two blue at the ventro-lateral sides and a red one at the medio-ventral side of the yolk plug. In the further development we will follow only the displacements of the blue marks.

As in *Tr. alpestris* 165 and 164, the invaginated material was considerably displaced to the dorsal side. In the early larva the blue mark lay in the nephrogenous cords and adjacent myotomes

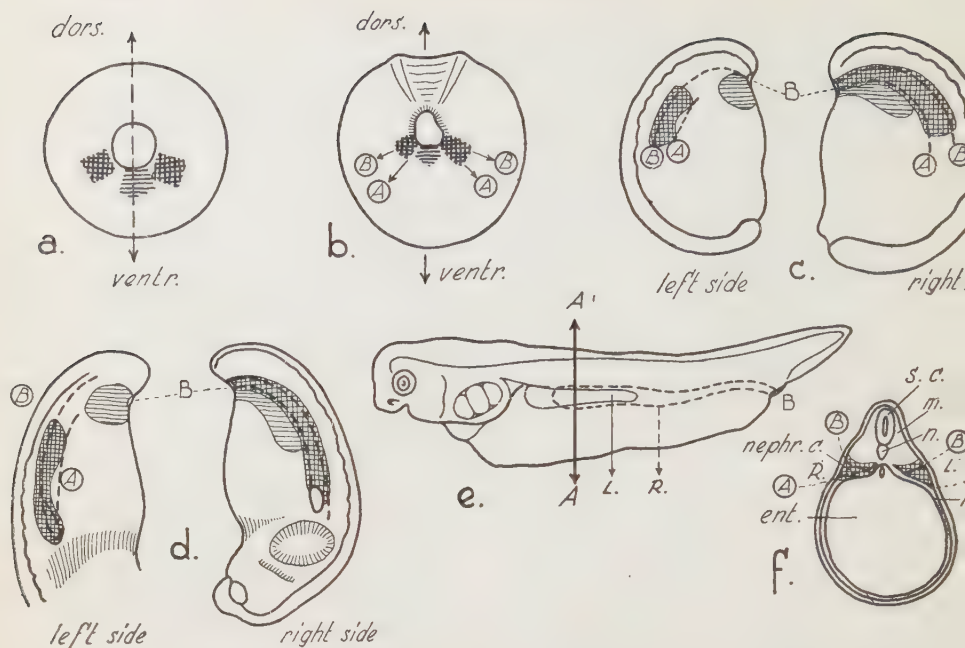


Fig. 34. Staining of medio-ventral and ventro-lateral blastoporal lips, *Tr. alpestris* 173. a) Application of marks at yolk plug stage, caudal view. b) External position of marks at slit-blastopore stage, caudal view, 21 hours. → A: Projection of ventral border, → B: Projection of dorsal border of presumptive nephrogenous cord mesoderm. c) Internal position of marks at early eye vesicle stage, left and right lateral views, 47½ hours. Dotted lines: Dorsal and ventral borders of nephrogenous cord mesoderm. d) Id., tail-bud stage, left and right lateral views, 67¼ hours. e) Id., early larva, left lateral view, 119 hours. Projection of left (continuous line) and right (broken line) dark marks. f) Transverse section of early larva along plane A-A', position of nephrogenous cord tissue. Enlargement a-e) ± 10×, f) ± 12×. L.pl.: lateral plate, neph. c.: nephrogenous cord.

and lateral plates. I determined the exact position of the dorsal and ventral borders of the nephrogenous cord with respect to the mark. Then, these borders were projected upon the staining mark on the sketch of the embryo at the slit-blastopore stage.¹⁾ In many of my experimental embryos I was able, in this way, to project the boundaries between the various parts of the meso-

¹⁾ During the end of gastrulation and the beginning of neurulation, the external marks did not deflect in a dorsal direction, but moved along the meridians towards the blastoporal lips. The organ-fields, projected upon the embryo at the slit-blastopore stage, occupy, therefore, the same position at the early yolk plug stage.

TABLE 7

No. of animal	Flank of animal	Angle between ventral median line and ventral border of presumptive nephrogenous cord cell-material	Angle between ventral median line and dorsal border of presumptive nephrogenous cord cell-material	Stage, at which vegetative margin of mark has been invaginated
173	L	47°	75° (—)	early-middle yolk plug
	R	50°	66° (—)	" " " "
141	L	58°	82° (—)	" " " "
	R		82° (—)	" " " "
189	R		82° (—)	" " " "
178	L	60°	90°	very early yolk plug
	R	45°	65°	late yolk plug
164	R	40°	58°	" " " "
142	L	58°	82°	early yolk plug
	R	48°		" " " "

derm upon the blastoporal lips, and to determine the angles which these lines made with the ventral median line. Table 7 gives the results.

Various mistakes may be made in the determination of these angles, so that the data obtained have but an approximate value. For instance mistakes may be made in the projection of the spherical embryo on a flat plane, in marking the position of the lines A and B and the ventral median line on the sketch of the embryo at the slit-blastopore stage, in the determination of the angles between the lines A and B and the ventral median line, etc. Notwithstanding these sources of errors, several conclusions can be drawn from this table.

As table 7 shows, the dorso-ventral extent of the presumptive lateral plate and nephrogenous cord mesoderm are fairly different in the various animals. On a closer examination it is striking that low values determined for the ventral border of the nephrogenous cord cell material correspond to low values for the dorsal border. Comparing the stage, at which the mark begins to be invaginated, with the values for the ventral and dorsal borders, it is evident that low values, correspond to later, and high values to earlier stages of invagination. This implies 1) that the dorsal and ventral borders of the nephrogenous cord do not coincide with the meridians in the old gastrula (yolk plug stage), but deflect ventrally,

and 2) that later invaginated parts undergo a stronger dorsal displacement than earlier invaginated parts. The ventral border of the presumptive nephrogenous cord, beginning, at the border of the vegetative field, at $\pm 60^\circ$ from the ventral median line (zone 1 fig. 35a), therefore deflects in ventro-cranial direction to $\pm 45^\circ$ (zone e fig. 35a). The dorsal border shows the corresponding values of $\pm 90^\circ$ and 60° - 70° .

Discussion.

As *Tr. alpestris* 165 clearly demonstrates, the movements of dorsal convergence appear in the invaginating mesoderm as soon as it has passed the lateral blastoporal lip. The same holds good for the ventro-lateral blastoporal lip (*Tr. alpestris* 154).

Tr. alpestris 156 shows that the mesoderm of the lateral plate and, probably, of the most caudal part of the blood island too, originates from the medio-ventral and latero-ventral external blastoporal lips. The other part of the blood-island must originate from the internal marginal zone.

The first invaginated mesoderm of the presumptive lateral plates never extends beyond the pronephros (cf. for instance *Tr. alpestris* 156). We may, therefore, conclude that the cranial border of the invaginated lateral plate mesoderm lies on a level with the pronephric rudiment, so that more cranial parts of the lateral plate must originate from the internal marginal zone. This is in entire agreement with an observation in the experiments on the vital staining of the entoderm. As fig. 13b, p. 64, shows, the external mesoderm, bordering the ventral half of the vegetative field, does not furnish the cranial margin of the invaginated ventral and lateral mesoderm, as the most cranial internal mesoderm is unstained. Therefore, the latter must have another origin. According to VOGT (1929), this material originates from the internal marginal zone which does not pass the blastoporal lips.

Tr. alpestris 170 shows that the later invaginated portions of the marginal zone undergo a much greater dorsal displacement than those which invaginate earlier. From this animal, but especially from *Tr. alpestris* 156, we may conclude that medio-ventral marks, situated at increasing distances from the ventral blastoporal lip at the yolk plug stage, consecutively stain more lateral and dorsal areas after invagination. In this way we can imagine that the broad triangular field, occupied by the ventral mesoderm after

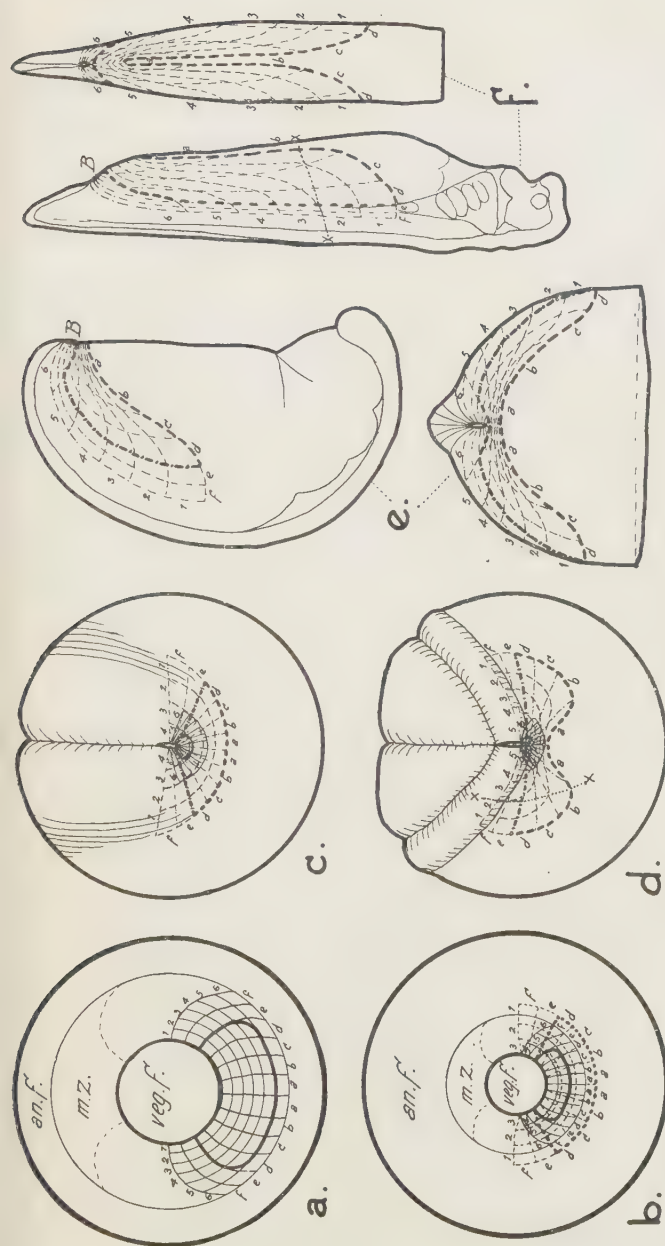


Fig. 35. Diagram of position of presumptive lateral plate and nephrogenous cord mesoderm. a) Yolk plug stage, caudal view. Ventral marginal zone divided into 6 animal-vegetative zones (1-6). Nephrogenous cord and lateral plate mesoderm in ventro-dorsal direction divided into 6 zones (a-f). Dark line: Outline of presumptive lateral plate mesoderm. Outer border of zone f: Dorsal boundary of nephrogenous cord mesoderm. Broken line: Dorsal boundary of presumptive myotomes. b) Middle yolk plug stage, caudal view, zone 1-3 invaginated. Internal position of mesoderm dotted. Dark dotted line: Outline of invaginated lateral plate mesoderm. c) Slit-blastopore stage, caudal view, zone 1-4 invaginated. Commencement of dorsal convergence. d) Early neurula stage, caudal view, zone 1-5 invaginated. X-X': Projected line of parts having passed the blastoporal lips simultaneously. Zone a-d extending laterad and decreasing mediad. e) Eye-vesicle stage, left lateral and ventral views, whole marginal zone invaginated. Continued alteration of presumptive lateral plate area, very considerable dorsal convergence. f) Early larva, ventral and left lateral views. Ventral extension of lateral plate mesoderm. X-X': Cf. fig. d. Enlargement a-e) $\pm 30\times$, f) $\pm 16\times$.

invagination, has been formed from successive narrow colour bands. Compare fig. 35a.

In my opinion, it is obvious that the rounded or triangular form of the mark in *Tr. alpestris* 188 and 189, and the bilobed form in *Tr. alpestris* 156, are successive stages. One day later the embryo *Tr. alpestris* 156 showed the first differentiation of the head. At that moment the two lobes of the blue mark had diverged still more, and had been displaced to the dorsal side. The continued invagination and the dorsal convergence altered the triangular form of the mark into a bilobed one.

The wide divergence of the two lobes of the mark in *Tr. alpestris* 188 at the tail-bud stage may find its explanation in the position of the mark. It was situated at some distance from the ventral border of the vegetative field at the gastrula stage, which implies that it had been invaginated rather late. It has, therefore, been displaced in dorsal direction over a fairly great distance.

On the ground of these vital staining experiments, the fate of the mesoderm of the presumptive lateral plates and blood-island during the gastrulation, neurulation and first differentiation of the larva may be summarized as follows: The cell material of the lateral plates of the trunk (from the pronephros to the anus) originates from the medio-ventral and latero-ventral external blastoporal lips. Each transverse strip of cells of the presumptive lateral mesoderm of the late gastrula (fig. 35a, zone 1-6) forms a narrow U-shaped band of cells, extending at the early tail bud stage in a cranio-caudal direction from the right side via the anus to the left side (fig. 35e and f). The first invaginating cell material (zone 1 fig. 35a) forms the most ventral and cranial U-shaped cell band (zone 1 fig. 35f). The last invaginating part (zone 5 fig. 35a) occupies the most dorso-caudal strip of cells of the lateral plate (zone 5 fig. 35f). The area, lying between the two lobes of the first invaginated lateral plate material, is occupied by the presumptive blood-island. This originates chiefly from the internal marginal zone. The last invaginating ventral mesoderm (during the neurulation) probably forms the caudal parts of the nephrogenous cords. The presumptive nephrogenous cord mesoderm, therefore, forms a horseshoe round the presumptive lateral plate mesoderm at the yolk plug stage (fig. 35a).

Up to the early slit-blastopore stage, the invaginated ventral mesoderm keeps a medio- and latero-ventral position (fig. 35b

and c¹)). At this stage, the movements of dorsal convergence begin to appear. In the following hours the first invaginated ventral and latero-ventral mesoderm (zone a-d fig. 35) extends considerably in lateral, and decreases in cranio-caudal direction, especially in the ventral median line (fig. 35d¹)). The original triangular shape of the invaginated ventral mesoderm, therefore, changes into a bilobed one. In this way the presumptive lateral plate mesoderm acquires a butterfly-shaped form. During the neurulation the presumptive lateral plate mesoderm borders on more lateral parts of the entoderm mass owing to the divergence of the two lobes. At the same time the area of the blood-island broadens considerably (fig. 35e).

This dorsal convergence proceeds till the incipience of the differentiation of the head and tail-bud; then it decreases. With the commencement of the stretching and elongation of the embryo contrary movements take place. Besides the cranio-caudal elongation of the lateral plate mesoderm its dorso-ventral extent now increases, chiefly by elongation in ventral direction.²) The blood-island stretches considerably in cranio-caudal direction, while its lateral extent rapidly decreases. During the elongation of the larva, this process continues. Finally, with the beginning of the active movements of the larva, the blood-island is changed into a narrow medio-ventral strip of cells (fig. 35f).

Comparison with the literature.

It is not my intention to give a complete survey of the literature concerning the displacements of the mesoderm of the caudal half of the trunk during gastrulation, neurulation and first differentiation of the larva.

The external displacements of the ventral to lateral marginal zone had been thoroughly investigated by VOGT (1926 and '29)

¹) A correction must be made in fig. 35b-d. I have not taken into consideration that the invagination of the lateral mesoderm begins at an earlier stage, and at first progresses more rapidly than that of more ventral mesoderm. Therefore the imaginary lines of cells, which pass the blastoporal lips simultaneously, in reality deflect dorsad to the blastopore; in fig. 35 they run parallel to the ring-shaped blastopore. In other words, in fig. 35d and f, the projection of the real invagination lines would correspond for instance to the line X-X' instead of to the line between zone 2 and 3. I will return to this question below, p. 101.

²) The dorsal border, however, is also displaced, first dorsally, and then medially between the entoderm mass and the ventral border of the myotomes.

and others. My results I found to agree very well with theirs, only with the exception that I did not observe any deflection from the radial displacement, in dorsal direction, as was described by SUZUKI (1929), in ventral direction by VOGT (1929). In my experiments the external marks were displaced exactly along the meridians from the early yolk plug stage till the very early neurula.

The dorsal convergence of the invaginated mesoderm was first observed and investigated by VOGT (1929). This dorsal convergence begins as soon as the invaginating mesoderm has passed the blastoporal lips.

VOGT (1929) explained the fact that the first invaginated parts have not been displaced as much as the later invaginated parts, as follows: The latter remain in the neighbourhood of the blastopore for a much longer time than the former, so that they are more strongly affected by the dorsal convergence.

The dorsal convergence also influences the ventral mesoderm, beginning at a somewhat later stage. The greater the distance from the mark to the ventral blastoporal lip, the later will be the invagination, the stronger will be the action of the dorsal convergence, and the more dorsal the final position of the mark.

VOGT (1929) already observed that the blood-island, the heart rudiment and the cranial part of the lateral plate originate from the internal marginal zone. The ventral internal marginal zone furnishes the blood-island, the ventro-lateral and lateral parts the heart rudiment and lateral plate of the cranial half of the trunk.

YAMADA (1938) made exhaustive investigations as to the internal and the invaginated cell-material of the marginal zone by implantation of very small stained grafts. He found that the lateral plate of the cranial and middle trunk region originates from the mesoderm, situated cranially, cranio-ventrally and ventrally to the pronephric rudiment at the slit-blastopore stage. The lateral plate of the caudal half of the trunk comes, however, from the mesoderm, situated medio-ventrally directly in front of the blastopore. He was the first to describe the dorsal and following ventral convergence of the presumptive lateral plate mesoderm during neurulation and stretching of the embryo.

According to VOGT (1929), YAMADA (1937 and '38) and others, the lateral external blastoporal lip furnishes the somites caudally from the third. The cell material of the three foremost somites must, therefore, originate from the internal marginal zone. The

external layer of the ventral to ventro-lateral marginal zone furnishes the lateral plate and the nephrogenous cord from circa the fourth or the fifth somite. Therefore the original dorso-ventral border line between the marginal zone and the vegetative field must take an oblique course after invagination, pointing ventro-caudally. During the stretching of the embryo this line acquires a more transverse position. Cf. fig. 35, line X.

After removing portions of the lateral marginal zone, SUZUKI (1929) found a reduction of the lateral plate. This observation does not agree with the results of VOGT, YAMADA and with my own.

After the changes made by PASTEELS (1939) in VOGT's "Anlagenplan" (1929), this agrees in many respects with my own sketch of the ventral to lateral marginal zone (fig. 35a). The whole ventral to ventro-lateral marginal zone is occupied by the presumptive organ fields of lateral plate and nephrogenous cord mesoderm. Moreover, the ventrad deflection of the line between presumptive nephrogenous cord and myotome organ fields corresponds to the ventrad oblique course of this line in PASTEELS' sketch. In the sketch of the tail formation in the Urodeles, NAKAMURA (1938) gives only the course of the ventral border of the myotomes. The position of this line corresponds fairly well to my own and PASTEELS' data, it merely ascends much farther to the dorsal side. The same holds good for the sketch of Miss BIJTEL (1929 and '31). The presumptive myotome field does not descend to the ventral marginal zone (VOGT (1929)), but ends in the ventro-lateral. Cf. fig. 35a.

On Miss BIJTEL's sketch of the presumptive organ fields in the early neurula, that part of the presumptive lateral plate mesoderm which is still situated externally, occupies a narrow crescent, extending in the lateral blastoporal lips. In agreement with NAKAMURA, I, however, found that the lateral plate mesoderm occupies a small horseshoe-shaped area round the blastopore.

Concerning the border between presumptive nephrogenous cord and lateral plate mesoderm, however, even PASTEELS gives but incomplete data. He records only the position of the presumptive Wolffian duct field along the ventral side of the 5th-7th somite (PASTEELS (1942)). The horseshoe-shaped form of the presumptive nephrogenous cord field round the presumptive lateral plate mesoderm is a new fact, and in this respect PASTEELS' "plan des ébauches" must be corrected.

Obviously most of the facts which I observed, were already known; their mutual relationship, however, had not yet been investigated sufficiently. I have endeavoured, in the preceding pages, to give a comprehensive view of the movements of the ventral mesoderm.

d. Defect-experiments of the mesoderm.

There exist in the larva close topographical relations between the germ cells, the genital ridges, the lateral plates, the Woffian ducts and the nephrogenous cords. It is, therefore, reasonable to suppose that the lateral plate and (or) the nephrogenous cord may play an important part in the formation of the germ cells. In order to investigate the significance of these parts in the formation of the germ cells, I made two experiments. In the first I completely removed the cell material of the presumptive lateral plates and nephrogenous cords (series A); in the second that of the presumptive somites (series B). (Only the mesoderm of the caudal half of the trunk has been removed, as the germ cells are localized in the posterior half of the body only.)

In order to remove this mesoderm completely without injury to the underlying entoderm, it was necessary to choose a developmental stage at which the mesoderm and entoderm have completely separated from each other in the field of operation. As this is not the case before the early neurula stage, I performed the operation at this stage.

Series 10a. Removal of the presumptive lateral plate and nephrogenous cord cell material.

Experiment and results.

We have first to determine the position of the cell material of the presumptive lateral plate and nephrogenous cord mesoderm at the neural plate stage. From the investigations of VOGT (1929), BIJTEL (1929), YAMADA (1937), NAKAMURA (1938), and others, we know that, at the neural plate stage, only the first twelve to fourteen body segments have been invaginated. The more caudal segments of the trunk ($\pm$ to segment 18) and the tail segments are still localized externally in the blastoporal lip. In order to remove the mesoderm of the future 7th to 18th body segments – the position of the “germinal region” – we have to extirpate both invaginated and external parts of the marginal zone.

In the preceding vital staining experiments, I have determined

the position of the presumptive somite, nephrogenous cord and lateral plate mesoderm at the early yolk plug and slit-blastopore stages. At the neural plate stage, this cell material has been displaced mainly along the meridians. Only a slight displacement to the dorsal side has to be taken into consideration on account of the appearance of the movements of dorsal convergence at this stage. The dorsal border of the presumptive nephrogenous cord cell material on the external marginal zone, therefore, is probably situated about 90° from the ventral median line. The invaginated lateral to ventral mesoderm is displaced dorsad over a larger extent. The dorsal border of the invaginated nephrogenous cord mesoderm will lie much more dorsally than in the external marginal zone. Regarding this internal position, the experiments of YAMADA (1938) and VOGT (1929) give more exact data. YAMADA records that at the early neurula stage the lateral border of the invaginated somite mesoderm (dorsal border of the nephrogenous cord) coincides with the outer line of the neural folds. At later neurula stages this border is displaced with respect to the neural folds in a lateral direction, as the movements of dorsal convergence of the neural plate surpass those of the underlying mesoderm.

Eggs of *Ambystoma mexicanum* were used. The early neurula lay with its caudal side turned upwards (fig. 36). With a glass needle the external and underlying cell layers were cut on either side of the blastopore along the outer border of the neural folds for a fairly short distance. Then cuts were made in ventral direction round the blastopore to the ventral median line. The invaginated

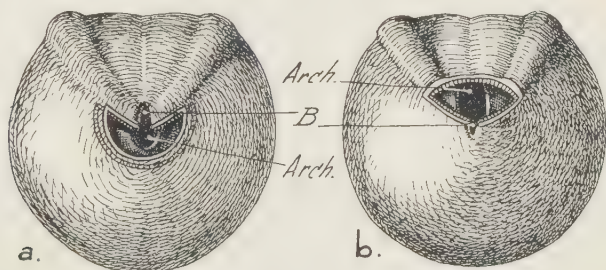


Fig. 36. a) Operation sketch of extirpation of presumptive lateral plate and nephrogenous cord mesoderm in early neurula of *Amb. mexicanum*, caudal view. Invaginated mesoderm removed to dotted line. b) Operation sketch of extirpation of presumptive myotomes from early neurula of *Amb. mexicanum*, caudal view. Enlargement $\pm 12\times$. Arch.: archenteron.

mesoderm was extirpated to a somewhat larger extent (hatched) than the external layer (white) by taking away a narrow strip of mesoderm under the margin of the ectoderm.

In order to prevent ambiguous results, I intentionally removed a field somewhat larger than the determined area of the presumptive ventral mesoderm. In this way the material of the presumptive nephrogenous cord, lateral plate, and caudal part of the blood-island of the posterior half of the trunk were completely removed. Small ventral parts of the posterior somites, ectoderm and mesenchyme of the tail, and the posterior trunk ectoderm were possibly also extirpated.

A few hours after the operation, as a rule, the wound had almost entirely closed. With the incipience of the longitudinal stretching of the embryo (fig. 37a) the ventro-caudal entoderm became visible again by the reopening of the wound.

The operated embryos formed a tail-bud, the ventral side of

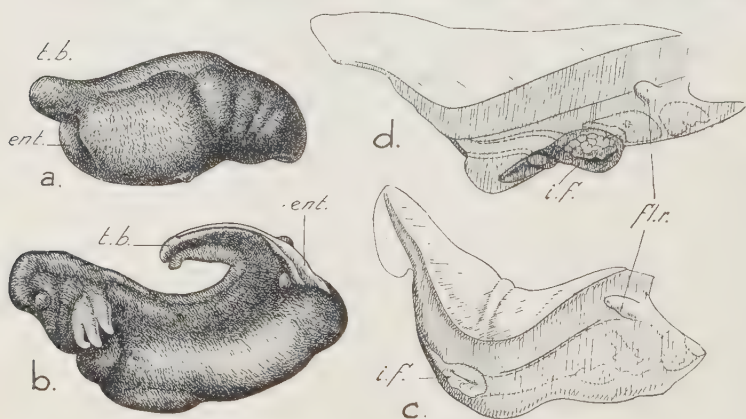


Fig. 37. Development of embryos after removal of ventral to lateral blastoporal lips at early neurula stage. a) *Amb. mexicanum* 480/1, early tail-bud stage, right lateral view, $1\frac{1}{2}$ day. b) *Amb. mexicanum* 478, late tail-bud stage, left lateral view, 4 days. c) and d) *Amb. mexicanum* 482 and 480/1, latero-ventral view, 13 days. Enlargement $\pm 10\times$. Large intestinal fistula (i.f.).

which had not been covered by the ectoderm; from the beginning it showed a slight bend to the dorsal side. These abnormalities were accentuated during further development. The anal fistula enlarged and the tail-bud bent far to the dorsal side. In the trunk, a part of the ventral and lateral mesoderm, and, in the tail, the whole ventral mesenchyme and ventral fin, were missing. The tail entoderm had, however, developed strongly and extended along the whole ventral side of the tail to its tip. Cf. fig.

37b. At later stages, the tail of the larva had not yet been covered entirely by ectoderm; the medio-ventral strip was formed by entodermal epithelium cells.

At the late tail-bud stage, but more especially at still later stages, large fistulae were formed in the ventral and lateral sides of the trunk. Several larvae showed large stomach fistulae, while in others the intestine in the left or right flank opened. Cf. fig. 37c and d. All the experimental animals of this series showed a reduced length of trunk and tail as compared with the controls. They were fixed 21 days after operation.

Table 8 gives the results of this experiment.

TABLE 8

Experimental animals of series 10a.			Controls of series 10.			Experimental animals of series 10b.	
Number of animal	Number of germ cells	Position of germ cells with regard to spinal ganglia	Number of animal	Number of germ cells	Beginning of fertile region with regard to spinal ganglia	Number of animal	Number of germ cells
473	1	sp. g. 9-10	483/7	189	sp. g. 8-9	499	144
474			484	147	" " 8-9	500	47
475/6			485/7	101	" " 9-10	501	130
477	1 and 5	" " 7-8 and ant. to sp.g. 12	486/7	105	" " 11-12	502	105
			488/90	74	" " 9-10	503	48
478	12	" " 9-10	489/90	148	" " 8-9	504	96
480			508/10	133	" " 9	505	116
482			509/10	99	" " 8-9		
7 ×	M = 3 m = ± 1.5		8 ×	M = 125 m = ± 13.5	M = sp.g. 9	7 ×	M = 98 m = ± 14.3

The table shows that 4 out of 7 experimental animals are completely sterile. Two others contain but a few germ cells, situated between the levels of the 9th and 10th spinal ganglion, and in the last one a small number of germ cells is present between the levels of the 7th and 8th and anterior to the 12th spinal ganglion. ¹⁾ The germ cells present showed a normal differentiation.

¹⁾ The spinal ganglia are used here, and also in later experiments, as being easily determinable landmarks of the segmentation of the body.

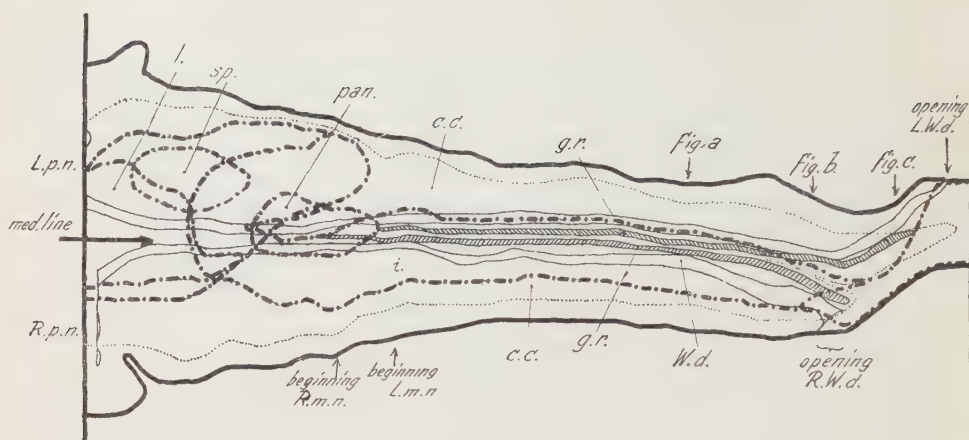


Fig. 38. Graphic reconstruction of trunk of *Amb. mexicanum* 482. Projection on frontal plane. Vertical enlargement $33\frac{1}{3}\times$. Horizontal: 1 section (8μ) = $\frac{1}{3}$ mm. Outlines of organs as in fig. 1, p. 13. Sp.: spleen, pan.: pancreas.

Another interesting fact of this series is the presence of rather well developed lateral plates (with coelomic cavities, germ ridges,

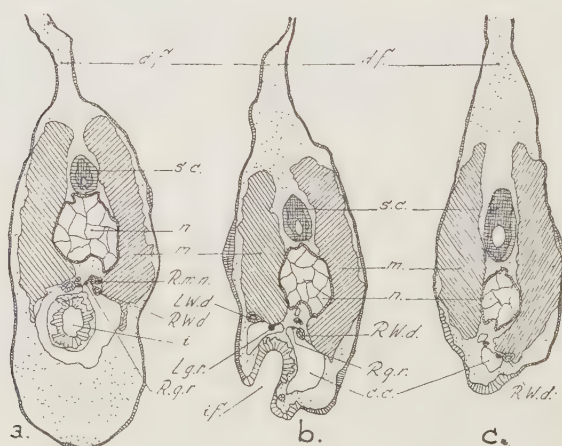


Fig. 39. Cross sections a, b and c through caudal trunk of *Amb. mexicanum* 482 (cf. arrows in fig. 38). Enlargement $33\frac{1}{3}\times$. G.r.: germ ridge, m.n.: mesonephros.

somatopleures and splanchnopleures), mesonephric blastemes and Wolffian ducts. In the graphic reconstruction in fig. 38 and in the three cross sections in fig. 39, we see the normal development of the intestine, coelomic cavities, Wolffian ducts and germ ridges in a large part of the trunk. Only the most posterior part of the trunk shows abnormalities. The intestine possesses a large fistula on the right and ventral side of the body; the right coelomic cavity, Wolffian duct and germ ridge end a short distance behind the beginning of this intestinal fistula. On the left side, these organs run more caudad, and end at a normal level.

Although the coelomic cavity has been reduced in the posterior trunk region, a well differentiated germ ridge has been formed at either side in the neighbourhood of the Wolffian duct. The absence of germ cells forms the sole difference with the structure of the normal germ ridges of the controls.

The position of the germ ridges is summarized in Table 9.

TABLE 9

Experimental animals				Controls	
Number of animal	Position of caudal end of coelomic cavity with regard to spinal ganglia	Structure of germ ridge	Caudal end of germ ridge with respect to spinal ganglia	Number of animal	Caudal end of fertile region with regard to spinal ganglia
473	about sp. g. 13	normal	about sp. g. 13	483/7	sp. g. 17-18
474	" " " 11-12	"	" " " 11-12	484	before " " 17
476/5	" " " 15	"	" " " 15	485/7	" " " 17
477	" " " 15	"	" " " 15	486/7	" " " 18
478	behind " " 15	"	behind " " 15	488/90	" " " 17
479/81	trunk hydropic			489/90	about " " 18
480/1	about sp. g. 16	"	about " " 16	508/10	before " " 18
482	" " " 14	"	" " " 14	509/10	behind " " 18
7 ×	M = behind sp. g. 14		M = behind sp. g. 14	8 ×	M = sp. g. 17-18

The table shows that in all cases the germ ridge has the same extent in caudal direction as the coelomic cavity. In the controls the germ ridge region begins on a level with the 9th spinal ganglion and ends between the 17th and the 18th ganglion. Comparing these values with the average position of the end of the germ ridges in the experimental animals (behind the 14th spinal ganglion) it is evident that, on an average, along $\frac{5}{8}$ of the germ ridge region well differentiated germ ridges have been formed.

Discussion.

Comparing the position of the germ cells present with that of the incipience of the fertile region in the controls – on an average behind the 9th spinal ganglion – the former, even in *Amb. mexicanum* 477, are situated at the beginning of the fertile region. Moreover, they lie in front of the area of operation. Therefore we may consider them as germ cells left behind during the operation. In these animals, evidently, the presumptive mesoderm of lateral plate and nephrogenous cord of the most cranial part of the “germinal region” has not been removed.

Thus we must conclude that the mesoderm of the presumptive lateral plates and nephrogenous cords have an important rôle in the formation or determination of the germ cells.

The experimental larvae generally show a partial development of the lateral plate, blood island, etc. in the posterior trunk region. In many cases even large fistulae have been formed in the ventral and sometimes in the lateral sides. Nevertheless, all organs are present. This is highly remarkable, as the entire cell material of the presumptive lateral plate and nephrogenous cord has been extirpated. Moreover, in the literature the opinion has been defended that the power of regulation in the mesoderm is already much reduced at the slit-blastopore stage. According to SUZUKI (1929), complete regulation is even already lacking after unilateral defects in the morula and early blastula stage. In the late blastula, the regulation is even more restricted. Finally, in the early gastrula the regulation of defects is limited to the cell material of the injured presumptive organ fields themselves. From the complete absence of organs, observed in several cases, SUZUKI concluded that the power of regulation has already disappeared at the early gastrula stage. This conclusion is, however, in contradiction to the constriction experiments of SPEMANN (1901-'03). He obtained fairly normal larvae from halves of early gastrulae. In my experiments, a considerable regulation must still have taken place after the early neurula stage.

As we shall see later on, the Wolffian duct grows out from the caudal part of the pronephric rudiment and, therefore, the presence of these ducts is to be expected in the animals of this series. This does not hold good with regard to the nephrogenous cord and the lateral plate. These parts must have been formed by regulation from other parts of the mesoderm. Their cell material must have been derived either from the somite mesoderm or from more cranial parts of lateral plate and nephrogenous cord, or from both. The following argument indicates that no displacement of mesoderm from cranial to more caudal segments has taken place. The few germ cells present are situated in the incipience of the fertile region. In the case of a cranio-caudal displacement, the germ cells, originating from the presumptive lateral plate mesoderm, cf. p. 144, would have been displaced too. This, however, does not agree with the facts. We must thus conclude that all this regulated cell material derived from more dorsal mesoderm (presumptive somite mesoderm). Displaced to more lateral and ventral positions it has formed nephrogenous cord and lateral plate tissue. The mesoderm of the

presumptive somites, situated in the external and invaginated marginal zone at the early neurula stage, has probably spread over the entire flank. This is to be explained only if we assume that this mesoderm had either not yet, or only "weakly", been determined at that stage. It must have been transformed into nephrogenous cord and lateral plate by altered inductive influences (entoderm, ectoderm, weakened influence of the notochord, etc.).

The interesting fact in this series is, however, that germ cells have not been formed in this regulated lateral plate tissue.

Series 10b. Removal of the presumptive somite cell material.

Experiment and results.

Fig. 36b, p. 103, shows the operation sketch. Two cuts were made along the outer border of the right and left neural folds through the superficial (ectodermal) and the deeper (mesodermal) cell layers. Then the caudal end of the neural plate with the underlying archenteron roof was extirpated without injury to the dorsal entoderm.

At first the wound closed, but at the early tail-bud stage the lips of the wound separated, and the dorso-caudal entoderm became visible again (fig. 40a). The ventral and lateral blastoporal lips developed normally, and the post-invagination of the mesoderm proceeded likewise. At later stages a small tail-bud was formed; the larvae showed a very irregular small short tail. Besides large intestinal fistulae in the middle trunk region all

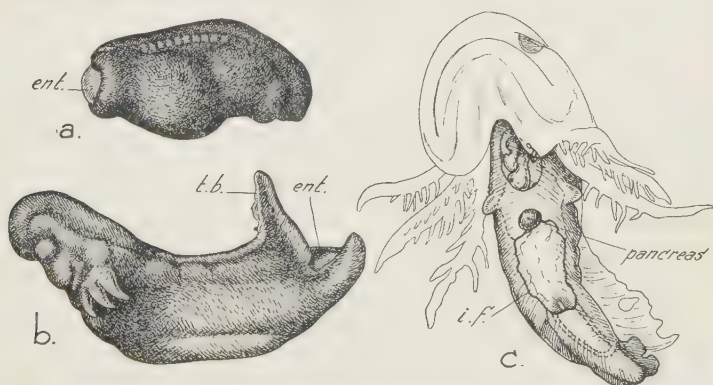


Fig. 40. Development of embryos after removal of latero-dorsal and dorsal blastoporal lips at early neurula stage. a) *Amb. mexicanum* 505, early tail-bud stage, right lateral view, $1\frac{1}{4}$ day. b) *Amb. mexicanum* 503, later tail-bud stage, left lateral view, 4 days. c) *Amb. mexicanum* 505, ventral view, 13 days.

Enlargement $\pm 10\times$

these animals showed considerable irregularities in the posterior part of the trunk and in the tail. In the posterior part of the trunk, the somites had been considerably reduced, while in the neighbourhood of the anus and in the tail they were often completely lacking. Owing to injuries to the caudal part of the notochord, this latter had developed very abnormally in the caudal part of the trunk and in the tail. It often showed many curvatures, and sometimes ended very irregularly. All the experimental animals were fixed 21 days after operation.

As Table 8 series 10b, p. 105, shows, the number of germ cells is hardly diminished. Only one animal was sterile. Here the intestine ended blindly in the middle trunk region. The sterility may therefore be explained by the lack of contact and interaction between the dorso-caudal entoderm and caudal mesoderm. Cf. p. 118. This animal has not been included in the determination of the average number of germ cells. The difference between the averages of the controls and the experimental animals of this series is not conclusive as $\frac{D}{m_D} = \frac{27}{19.3} = 1.4$.

As far as the coelomic cavities had been formed normally – in the neighbourhood of a large fistula the coelomic cavity was, as a matter of course, missing – normal germ ridges had been developed along the Wolffian ducts. In some cases a large lateral intestinal fistula had displaced the Wolffian duct and germ ridge to the lateral, or even to the ventral side of the flank.

Discussion.

From series 10b we must conclude that the presumptive somite mesoderm can be spared in the formation and development of the germ cells.

In the posterior part of the trunk small myotomes had developed, especially in the cranial segments of the area of operation. A slight regulation of somite mesoderm from more lateral mesoderm must have taken place.

HOLTFRETER (1933) observed that ectoderm and entoderm do not join each other without interjacent mesoderm. The retraction of the wound edges after the closing of the wound may, therefore, be caused in series a by the defect of the ventral and lateral mesoderm (and ectoderm). In series b the defect in the dorso-caudal mesoderm and the neural plate may be the reason of this retraction of the edges of the wound.

Ambystoma embryos readily adhere to glass or cellophane and, therefore, often show fistulae after temporary adhesion to the bottom of the ZIMMERMANN'S dishes. In the present experimental series this phenomenon was observed so frequently, however, – notwithstanding a bottom layer of agar-agar –

that other factors must have been in play. It may be caused by local defects in the interjacent mesoderm, whereby the ectoderm and entoderm come into contact with each other. Just as in the mouth field, such a state cannot last, and an opening must soon appear. Fig. 37d, p. 104, shows such a large intestinal fistula. In series b, this explanation does not hold good, since many animals of this series exhibit large intestinal fistulae, notwithstanding the presence of an undisturbed lateral plate and nephrogenous cord mesoderm. A satisfactory explanation cannot be given here.

As all the experimental animals of series a show less length of trunk and tail than the controls, the absence of the lateral and ventral mesoderm has evidently influenced the elongation of the larva. In series b, the notochord, myotomes and spinal cord are defect in the tail and posterior part of the trunk, which is the reason of the lessened length of these parts here.

The dorsal curvature of the tail in series a may be explained by a disturbance of the equilibrium between the dorsal and the ventral tail mesoderm and ectoderm, since the ventral are absent for the greater part. During the stretching of the tail, the notochord may be less counterbalanced on the ventral than on the dorsal side, whereby the tail may be bent to the dorsal side. In series b the somites and notochord are partly lacking in the tail, so that the stretching of the tail is often very irregular.

e. Discussion of the defect experiments of entoderm and mesoderm.

In my opinion this series of experiments has brought the problem of the origin of the germ cells one step nearer to its solution. It has become clear that not only the dorso-caudal entoderm, but also the ventral mesoderm influences the formation of the germ cells. The question arises as to what is the significance of these two parts?

We can distinguish between three possibilities.

1) All the germ cells originate from the dorso-caudal entoderm; for their differentiation, however, an inductive action from the mesoderm is necessary.

2) The germ cells originate partly from the dorso-caudal entoderm, partly from the lateral and ventral mesoderm.

3) All the germ cells originate from the lateral to ventral mesoderm; their differentiation is dependent on an induction by the entoderm.

Extirpation of a part of the dorso-caudal entoderm causes a reduction in the number of germ cells. The germ cells present are localized normally along the reduced fertile region. Parallel with a decreasing number of germ cells the differentiation of the germ cells and the length of the fertile region also decrease.

Removal of the presumptive lateral plate and nephrogenous cord mesoderm, on the other hand, often

causes a total absence of germ cells. Cells which remain, if any, are situated in the most cranial part of the "germinal region", and always show a good differentiation.

I will successively discuss these facts in the light of the three possibilities mentioned above.

1) In the first, the germ cells, originating from the dorso-caudal entoderm, must be determined by an inductive action of the ventral to lateral mesoderm. Removal of a portion of the entoderm implies a reduction of the presumptive germ cell material. The extirpation of the lateral to ventral mesoderm will, however, cause a diminution in the induction centre, so that few or no germ cells can differentiate. The localization and differentiation of the germ cells left behind, however, argues against this interpretation. After removal of part of the caudal entoderm, irregularities in the localization of the germ cells along the "germinal region" may be expected. Moreover, there is no reason to expect an inferior differentiation of the germ cells, since the inductive action has not been influenced by the operation. On the other hand, the incomplete removal of the lateral to ventral mesoderm will cause a considerable diminution in the inductive centre, by which the inductive action will be reduced. Thus, here, a poor differentiation of the germ cells may be expected. Moreover, it is obvious that the germ cells, which will nevertheless differentiate, will be found in the centre, and not in the most cranial part of the "germinal region".

The first hypothesis, therefore, does not tally well with the facts revealed by the experiments.

2) The second supposition does not hold. In that case, the extirpation of the lateral to ventral mesoderm cannot cause the observed complete sterility of the animals.

3) Only the third hypothesis agrees completely with the facts. The arguments against the first hypothesis plead in favour of this interpretation. The removal of the dorso-caudal entoderm implies a decreased inductive action, which will cause a reduction in the number of germ cells, together with a poorer differentiation of them. The germ cells formed will be restricted to the middle part of the "germinal region", since only in the middle part of the inductive centre will the action be strong enough to determine the differentiation of the germ cells. The extirpation of the lateral to ventral mesoderm means the removal of the presumptive germ cell material itself, and may, therefore, cause complete sterility.

Cells of the presumptive germ cell material, left behind after the operation, will undergo a normal inductive action and will differentiate normally.

In the light of these considerations the third hypothesis is the most probable.

On the basis of this hypothesis, I will now consider the significance of the various parts of the mesoderm as a probable source of origin of the germ cells. As we have seen above, the extirpation of the presumptive somite material has no influence upon the formation of the germ cells. Only the cell material of the lateral plates and nephrogenous cords can, therefore, contain the presumptive germ cells. Which of the two parts (lateral plate or nephrogenous cord, or both) does contain them, cannot yet be determined.

This series of experiments has revealed other interesting facts concerning the problem of the formation of the so-called secondary germ cells. In several cases, large parts of the lateral plate and nephrogenous cord have been regulated from the dorsal mesoderm without differentiation of (secondary) germ cells. I might imagine one reason or circumstance only which might explain the lack of differentiation of secondary germ cells (if they really exist) from one of the various tissues of the mesoderm; *viz.* that the sec. p. germ cells can differentiate only after an induction from the p.p. germ cells. Then we must, moreover, assume that individual p.p. germ cells can induce the formation of sec. p. germ cells from adjacent mesodermal cells. The differentiation of only a few isolated germ cells, or even of one, after incomplete removal of the presumptive lateral plate and nephrogenous cord mesoderm is, however, difficult to reconcile with such a formation. Cf. further the heteroplastic transplantation experiments of the mesoderm, p. 144. In my opinion, we may therefore conclude that it is very improbable, at least from the early yolk plug stage till the stage of fixation (stage 43 GLEASNER) that differentiation of secondary germ cells occurs in the Urodeles.

The fact that p.p. germ cells do not appear in the regulated lateral plate and nephrogenous cord, also indicates that the p.p. germ cells probably do not differentiate from common mesodermal cells. It argues in favour of the opinion that the germ cells may be present in a predetermined state among the presumptive lateral plate and nephrogenous cord cells already at the early yolk plug stage. Cf. further p. 146.

These are highly interesting and important considerations, which, however, have still to be tested.

f. Separation of the germ layers.

Series 11. Removal of the whole entoderm at the early neurula stage.

Results.

The method of operation and the development and differentiation of the embryos and larvae have already been described in the first part of this paper (cf. p. 10-11). Here I will merely record the number and position of the germ cells in these experimental animals.

TABLE 10

<i>Tr. taeniatus</i> V				<i>Tr. taeniatus</i> VIII				<i>Tr. taeniatus</i> XI			
Experimental animals		Controls		Experimental animals		Controls		Experimental animals		Controls	
Numb. of animal	Numb. of germ c.	Numb. of animal	Numb. of germ c.	Numb. of animal	Numb. of germ c.	Numb. of animal	Numb. of germ c.	Numb. of animal	Numb. of germ c.	Numb. of animal	Numb. of germ c.
375	39	I	223	371	71	I	289	384	150	I	315
367	55	II	89(?)	373	10	II	197	383	57	II	528
369	82			374	44						
370	12			368	9						
372	6			382	34						
399	73			380	0						
				381	23						
6 × M = 44,5 (29%)		2 × M = 156		7 × M = 27 (11%)		2 × M = 243		2 × M = 104 (25%)		2 × M = 421	

The larvae developed from eggs of three *Tr. taeniatus* females. The animals were reared till 32 days after operation. Table 10 gives the results of this experimental series.

The total average of the number of germ cells of the experimental animals amounts to 44 ($m = \pm 10,2$), being 16% of the general average of the controls: 273 ($m = \pm 60,3$). The diminution in the germ cell number is conclusive, notwithstanding the

small number of controls: $\frac{D}{m_D} = \frac{229}{61.2} = 3.7$.

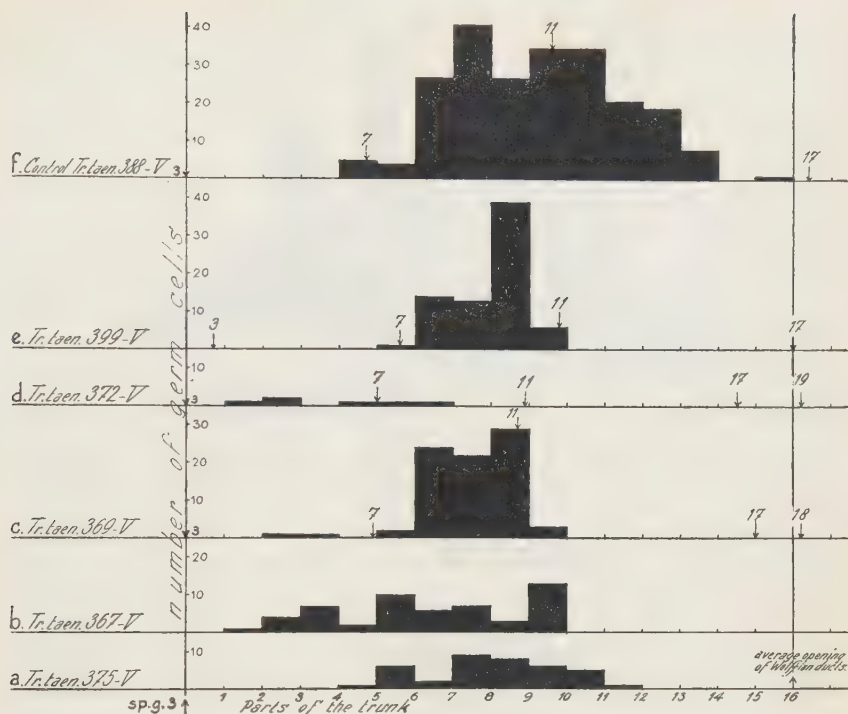


Fig. 41. Diagrams of germ cell distribution of experimental and control animals from *Tr. taeniatus* V. Horizontal: Trunk (from third spinal ganglion or, in default of this, from end pronephros to opening of Wolffian ducts in ectodermal proctodaeum) divided into 16 equal parts. Vertical: Number of germ cells. Position of third, seventh, eleventh, seventeenth, etc. spinal ganglion indicated in diagrams. a) *Tr. taeniatus* 375. b) *Tr. taeniatus* 367.

c) *Tr. taeniatus* 369. d) *Tr. taeniatus* 372. e) *Tr. taeniatus* 399. f) Control *Tr. taeniatus* 388.

In all the experimental animals fairly well differentiated germ cells were found (one animal only was probably sterile). Sometimes a few degenerating or degenerated cells, probably representing germ cells, were present.

The diagrams show the interesting fact that in every case the germ cells present are localized in the middle trunk region or, in other words, in the cranial part of the "germinal region" only. Cf. figs. 41, 42 and 43. With regard to the distribution of the germ cells present, the following may be remarked: In the animals with a relatively high number of germ cells, viz., the nos: 369, 399 and

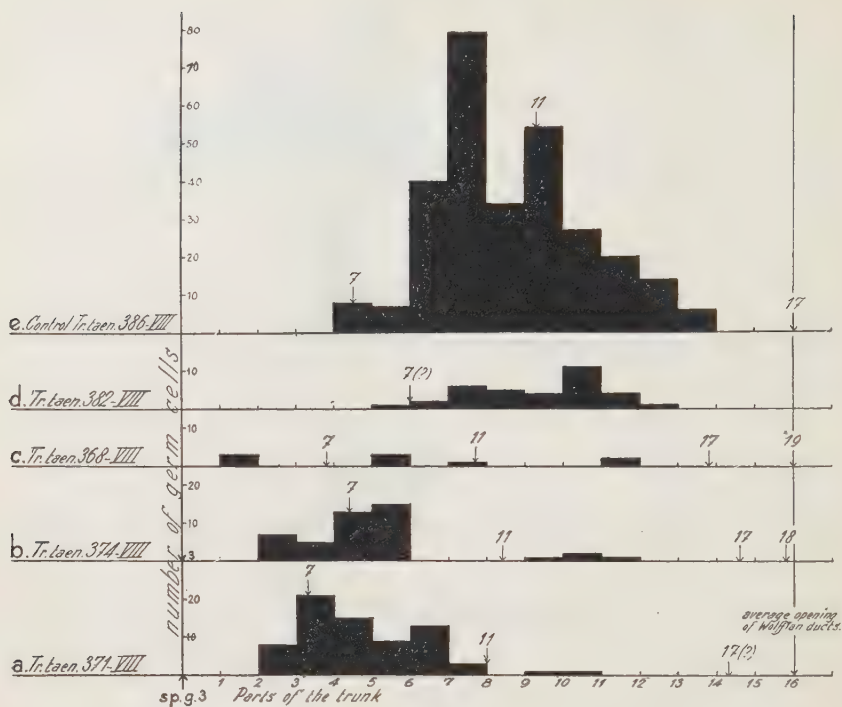


Fig. 42. Id., *Tr. taeniatus* VIII. a) *Tr. taeniatus* 371. b) *Tr. taeniatus* 374. c) *Tr. taeniatus* 368. d) *Tr. taeniatus* 382. e) Control *Tr. taeniatus* 386.

384, the distribution of these cells corresponds fairly well to that of the controls as far as the level of the 11th spinal ganglion. Caudally to this ganglion, the number of germ cells soon falls to zero in these experimental animals. In the animals 383, 371 and 374, the number of germ cells almost agrees with that of the controls to the 8th or 9th ganglion, and then decreases. Finally in the animals 367, 375, 372, 368 and 382 the numbers of germ cells are very low, and no connection with the distribution of the germ cells of the corresponding controls can be seen. Of the animals 370, with 12, 373 with 10, 380 with 0 and 381 with 23 germ cells no diagrams could be made on account of the marked curvature of the trunk. In every case the few germ cells present were localized in the cranial part of the "germinal region".

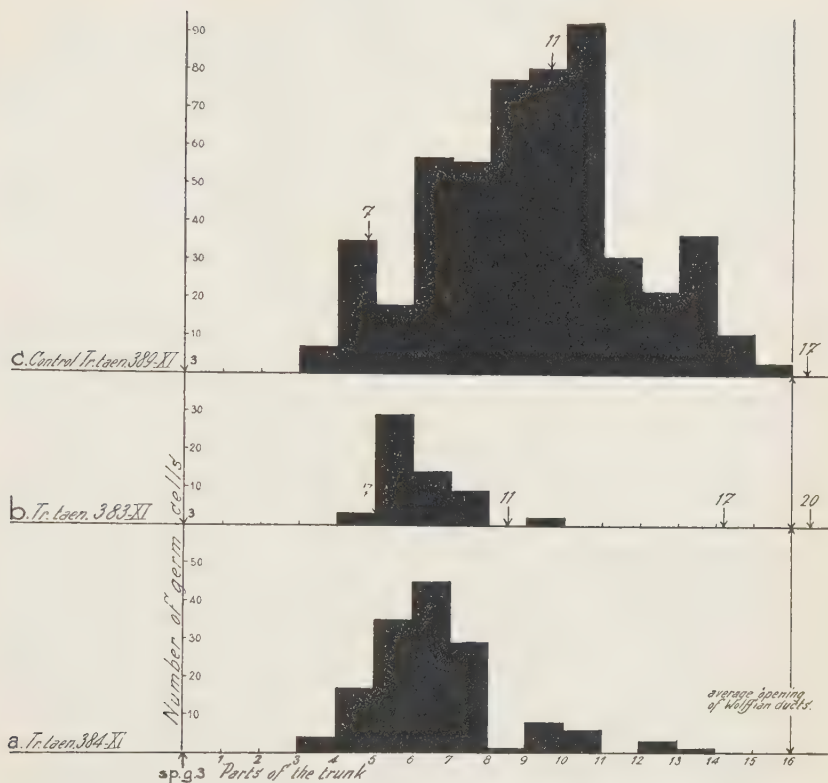


Fig. 43. Id., *Tr. taeniatus* XI. a) *Tr. taeniatus* 384. b) *Tr. taeniatus* 383. c) Control *Tr. taeniatus* 389.

Discussion.

Some degenerating germ cells having been observed, it was possible that the original number of germ cells had been somewhat larger than the number determined. The animals with a small germ cell number, however, do not show more but, on the contrary, fewer degenerating cells than the animals with larger number of germ cells. The low values have, therefore, not been caused by the degeneration of a large number of germ cells. The real cause must be another.

To give a satisfactory explanation of these facts, I will call in mind observations of VOGT (1929), YAMADA (1937), BIJTEL (1929) and others; viz., that at the early neurula stage only the meso-

derm of the cranial segments of the trunk has been invaginated, and that the presumptive mesoderm of the posterior part of the body is still situated in the external cell layer round the blastopore. According to Miss BIJTEL, only the twelve to fourteen cranial segments of the presumptive somites have been invaginated at the early neurula stage. The invaginated part of the ventral mesoderm is, probably, even smaller, so that only eleven or twelve

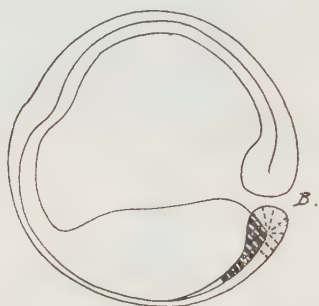


Fig. 44. Diagrammatic medio-sagittal section through early neurula of a Urodele. Invagination of ventral mesoderm; invaginated parts of presumptive lateral plate mesoderm (dark) in contact with entoderm. Enlargement $\pm 25\times$.

segments have passed the blastoporal lip. We can observe a striking similitude of the position of the germ cells with the above-mentioned facts. It may therefore be concluded that in these entoderm-free animals only that part of the mesoderm that has been invaginated at the time of operation, contains germ cells. The other part, which was at that moment still situated outside the blastopore, is completely sterile. Thus, this experimental series again furnishes a strong argument in favour of the supposition of the necessity of an inductive action preceding the differentiation of the germ cells. Only that part of the mesoderm contains germ cells which has come into interaction with the dorso-caudal entoderm (fig. 44).

On the other hand, it can be imagined, however, that the germ cells originated in the entoderm, but that some of them had already migrated into the mesoderm before the removal of the entoderm. Therefore, even this experiment does not yet prove whether the dorso-caudal entoderm or the lateral to ventral mesoderm forms the source of the p.p. germ cells. The other hypothesis, however, according to which the mesoderm is the source

of the germ cells, and the entoderm forms the inductive centre, is much more obvious and much simpler.

Finally, I would call attention to the fact that the germ cells present are situated in the dorsal somatopleure, scattered among the mesenchyme cells in the neighbourhood of the Wolffian ducts. Germ ridges have not been formed in these entoderm-free animals. I will return to this fact in the third part of this paper.

g. Heteroplastic transplantation.

a) General consideration.

The defect-experiments of entoderm and mesoderm indicate that the presumptive lateral plate and (or) nephrogenous cord mesoderm contain the presumptive germ cells, and that an inductive influence is exercised by the dorso-caudal entoderm. If it were possible to obtain experimental animals in which the cells of entodermal origin could be distinguished from those of mesodermal origin, this problem might be solved beyond all question. With this in mind, I sought the solution in heteroplastic transplantation experiments.

In the literature many heteroplastic transplantations have been described between *Triturus* and *Ambystoma* species, as the size of the *Ambystoma* cells is, in general, much larger than that of the *Triturus* species. (RAVEN (1931-'33)). An exact determination of the sizes of the germ cells in the two genera – I used *Tr. taeniatus* and *Amb. mexicanum* – showed, however, that there is hardly any difference.

In order to obtain a clear picture of the average, and variability, of the size of the germ cells, I determined variability curves in the two species. In cross sections the outlines of nucleus and cell of a large number of germ cells were transferred with the aid of a Zeiss drawing glass (enlargement 900 $\times$) on to white cardboard of a very homogeneous composition and thickness (experimentally tested). The figures were cut out, and finally weighed. In order to prevent an involuntary selection, the values have been determined of every germ cell, localized over a certain length of the fertile region. Thus, 448 germ cells of 15 controls and 4 experimental animals (transplantation of the central blastomeres at the early gastrula stage) of *Tr. taeniatus* and 647 cells of 6 *Amb. mexicanum* controls have been determined. Fig. 45 shows that the two curves almost entirely overlap. Only the largest germ cells of *Ambystoma* fall outside the *Triturus* curve. The size of the

germ cells, therefore, is quite useless for distinguishing the germ cells of the two species.

After this negative result I looked for other species characters, which differ in two or more Urodele species. The shape of nucleus and cell, the staining of the cell compounds, the shape of plastosomes, etc. exhibit a very great variability, and hardly differ among the various species. The only reliable difference was found in the quantity of pigment granules. Most of the Urodeles

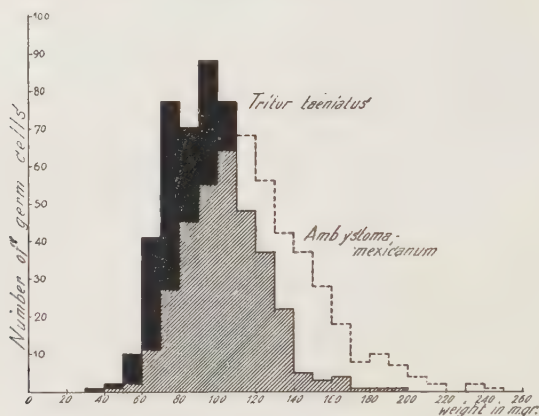


Fig. 45. Variability polygons of size of germ cells in *Tr. taeniatus* (continuous line) and *Amb. mexicanum* (dotted line). Horizontal: Size of germ cell nucleus, expressed in weight of cardboard, upon which it was projected. Vertical: Number of germ cells.

contain much embryonic pigment in every body cell, except *Tr. cristatus*, which has very little pigment in its tissues. Its germ cells, too, contain hardly any pigment granules.

I determined the number of pigment granules in 70 germ cells of *Tr. cristatus* and in about 150 germ cells of *Tr. taeniatus*, taken at random. Among 70 *cristatus* germ cells five contain more than 10 pigment granules, maximally 17, while the general average amounts to 4. Out of 70 germ cells 33 contain only two pigment granules or fewer (fig. 46). Other *cristatus* larvae give still lower values. The other tissues of *Tr. cristatus* also yield but few pigment granules, only the intestinal epithelium has more pigment. *Tr. taeniatus* cells, on the other hand, contain much more pigment. In most of the germ cells the number could not be determined. Therefore, I could only divide the germ cells into four groups,

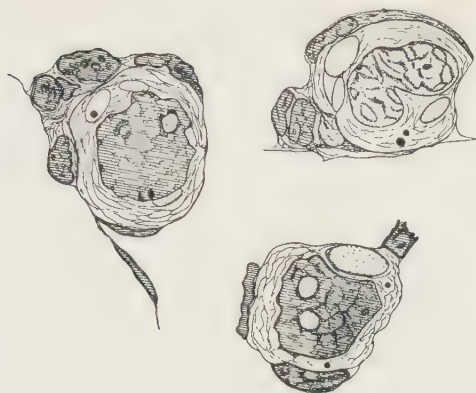


Fig. 46. Three germ cells of *Tr. cristatus*, taken at random with adjacent peritoneum cells. Enlargement $\pm 900\times$. Stained with DELAFIELD hematoxylin. Quantity of pigment.



Fig. 47. Four germ cells of *Tr. taeniatus*. a) Group I, very few pigment granules. b) Group II, more pigment granules. c) Group III, still more pigment granules. d) Group IV, very many pigment granules. Enlargement $\pm 1350\times$. Fixation in CHAMPY (fat spherules black). Stained with DELAFIELD hematoxylin.

the first containing very few, the second more, the third still more and the fourth a great many pigment granules (cf. fig. 47 a, b, c and d). Among 150 germ cells of *Tr. taeniatus* only 3 belong to the first group. The third group contains the largest number, while the second and fourth groups are composed of somewhat fewer cells than the third. The three cells of the first group contain 12, 19 and 20 pigment granules, respectively. The cells of nephrogenous cord, lateral plate and intestinal epithelium, as a rule, contain a great many pigment granules. The amount of pigment in the other *Urodeles* species hardly differs from that of *Tr. taeniatus*. *Tr. alpestris* usually contains more pigment, while the colour is darker, and *Amb. mexicanum* about the same quantity as *Tr. taeniatus*. Its pigment is very dark.

These values show that the germ cells of *Tr. cristatus* and *Tr. alpestris* (or *Amb. mexicanum*) are distinguishable from each other in almost every case. The following heteroplastic transplantations were, therefore, carried out between *Tr. cristatus* and *alpestris*, and in a few cases between *Tr. cristatus* and *Amb. mexicanum*. Compare SPEMANN and SCHOTTÉ (1932), ROTMANN (1931 and '33), etc.

b) Series 12. Heteroplastic transplantation of the whole entoderm at the early neurula stage.

Experiment and results.

The method of operation (fig. 48) may be described in a few words, as the removal of the whole entoderm has been fully recorded in series 1, p. 10 and 11. In the early neurulae of *Tr. alpestris* and *cristatus*, for instance, the whole entoderm is separated from the surrounding ecto-mesodermal envelope. Mesodermal cells adhering to the entoderm mass, and entodermal cells adhering to the inner side of the ecto-mesodermal envelope, are carefully removed. Then the round entoderm mass of *Tr. alpestris* is grafted into the ecto-mesodermal envelope of *Tr. cristatus*.¹⁾ The wound

¹⁾ One of the difficulties met with in this experiment is the shrinkage of the ecto-mesodermal envelope. Therefore it is always necessary to graft the entoderm of a smaller neurula into the ecto-mesodermal envelope of a larger one. The transplantation of the entoderm has been carried out as follows (in the direction of the arrows): (*Tr. taeniatus* →) *Tr. alpestris* → *Tr. cristatus* → *Amb. mexicanum*. Therefore the inverse transplantation (*Tr. cristatus* → *Tr. alpestris*) cannot be carried out in the same way, as the *cristatus* entoderm is too large in respect to the *alpestris* ecto-mesoderm. In this case the *cristatus* entoderm has been cut transversely, and only the posterior part of the entoderm has been grafted.

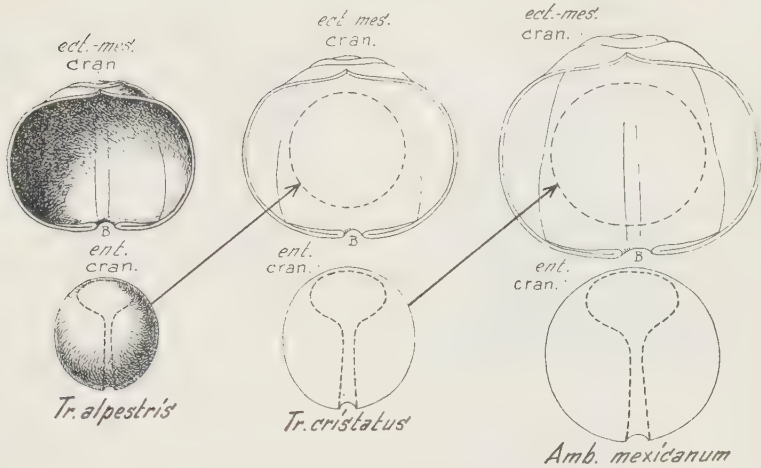


Fig. 48. Operation sketch of heteroplastic transplantation of whole entoderm between *Tr. alpestris*, *Tr. cristatus* and *Amb. mexicanum* (L. to R.). Above: Ecto-mesodermal envelope, ventral view. Broken line: Projected entoderm of *Tr. alpestris* in ecto-mesodermal envelope of *Tr. cristatus*, etc. Below: Entoderm mass, ventral view. Broken line: Projected dorsal borders of entoderm. Enlargement $\pm 10\times$.

margins of the ecto-mesodermal envelope are folded together round the grafted entoderm mass to the ventral median line. In this way the entoderm is again surrounded by ecto- and mesoderm. In order to prevent curling and shrinkage of the wound margins, the operation must be carried out as rapidly as possible.

In this operation the use of very early neurulae is of great importance. In older neurulae shrinkage of the ecto-mesodermal envelope is more considerable on account of the formation of the neural folds. Moreover, the separation of the germ layers causes more difficulty, and the wound-margins of the ecto-mesodermal envelope incline to curl outwards, which hinders the closing of the large wound.

As the operation presents many technical difficulties and the *cristatus* egg material always contains many abnormal eggs, but few experimental animals reached the desired age in this series.

In these animals the margins of the wound grew together. On the ventral side slight abnormalities, such as an irregular branchial cavity, two heart rudiments, and intestinal fistulae, often appeared. Nevertheless the embryos developed into rather nor-

TABLE 11

<i>Tr. cristatus</i> ecto-mesoderm <i>Tr. alpestris</i> entoderm		<i>Tr. alpestris</i> ecto-mesoderm <i>Tr. cristatus</i> entoderm		<i>Amb. mexicanum</i> ecto-mesoderm <i>Tr. cristatus</i> entoderm		<i>Tr. alpestris</i> (III) ecto-mesoderm <i>Tr. taeniatus</i> (VIII) entoderm	
Number of animal	Number of <i>crist.</i> g. c.	Number of animal	Number of <i>alp.</i> g. c.	Number of animal	Number of <i>Amb.</i> <i>mex.</i> g. c.	Number of animal	Number of <i>alp.</i> (?) g. c.
359/60 356/8 356/7	62 101 (6 alp) 100	342/3 345	68 187	346/7 348/9 350/1	67 106 41	377 378	70 103
3 × M = 88		2 × M = 128		3 × M = 71		2 × M = 87	
Controls of <i>Tr. cristatus</i>		Controls of <i>Tr. alpestris</i>		Controls of <i>Amb. mexicanum</i>		Controls of <i>Tr. alpestris</i> (III)	
359/61 362/3	331 450	364 365 366	141 202 195	352/3 354/5	285 232	391 392	191 191
2 × M = 391		3 × M = 179		2 × M = 258		2 × M = 191	

mally structured larvae. They were fixed 24 days after operation.

The microscopic analysis of the experimental animals furnished the following facts. The trunk was of fairly normal structure. The somatopleures and splanchnopleures had developed normally, and germ ridges had been formed. The entoderm had formed a normal intestinal epithelium, only the caudal part frequently showed an irregular, considerably enlarged anal opening in consequence of partial closing of the wound-margins of the ecto-mesodermal envelope. Intestinal fistulae on the ventral side of the trunk, explainable in the same way, were present in most cases.

All the experimental animals contained germ cells, having the species characters of the ecto-mesodermal envelope. The germ cells showed a good differentiation. Only one animal possessed 4 germ cells (absolute number) of the species which furnished the entoderm.

Table 11 shows that the number of germ cells in the experimental animals is only a fraction of that of the corresponding



Fig. 49. Diagrams of germ cell distribution in experimental animals *Tr. cristatus/alpestris* and control *Tr. cristatus*. Transplantation of whole entoderm. Horizontal: Trunk (from third spinal ganglion to ending of Wolffian ducts into ectodermal proctodaeum) divided into 16 equal parts. Vertical: Number of germ cells. Position of third, seventh, eleventh, seventeenth, etc. spinal ganglion indicated in the diagrams. a) *Tr. cristatus/alpestris* 359/60. Dark: Number of *cristatus* germ cells. b) *Tr. cristatus/alpestris* 356/8. Dotted: Number of *alpestris* germ cells. c) *Tr. cristatus/alpestris* 356/7. d) Control *Tr. cristatus* 362/3.

controls, viz., *Tr. cristatus* (host)/*alpestris* (grafted entoderm) 22.5%; *Tr. alpestris/cristatus* 71.5%; *Amb. mexicanum/Tr. cristatus* 27.5% and *Tr. alpestris/taeniatus* 45.5%. The germ cell numbers are larger in this series than in the experimental series 11, in which the whole entoderm has been removed. The most interesting fact is, however, the position of the germ cells. Cf. figs. 49-52. Many of the experimental animals show a sterile caudal

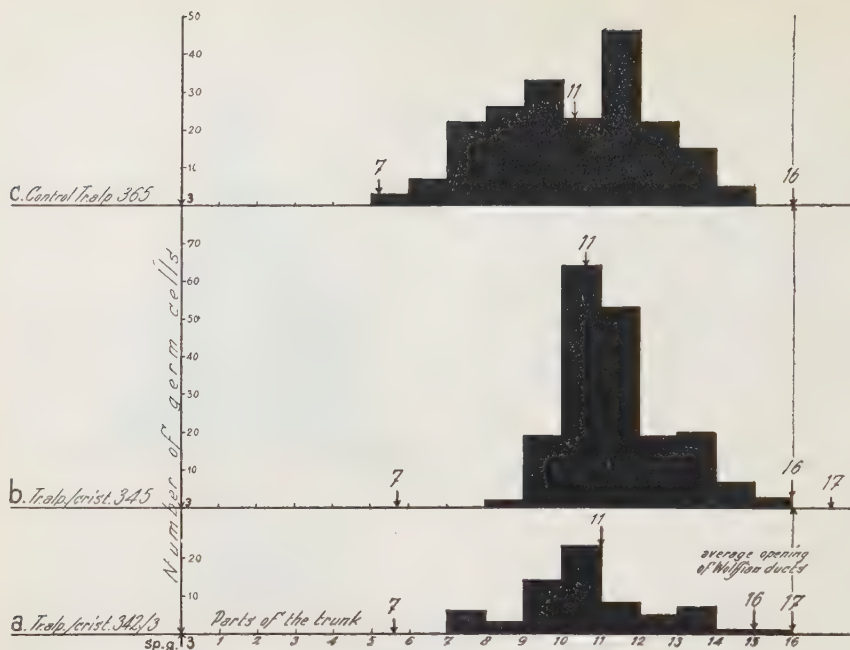


Fig. 50. Id., experimental animals *Tr. alpestris/cristatus* and control *Tr. alpestris*. a) *Tr. alpestris/cristatus* 342/3. b) *Tr. alpestris/cristatus* 345. c) Control *Tr. alpestris* 365.

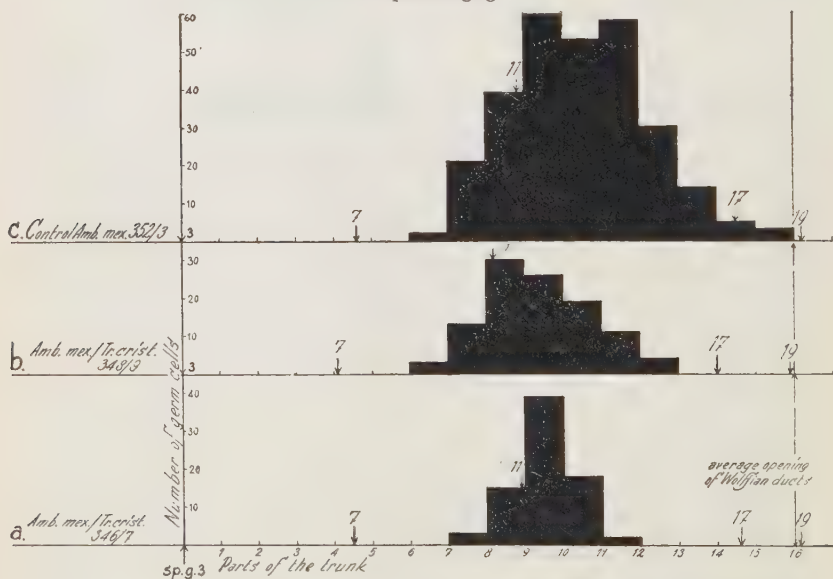


Fig. 51. Id., experimental animals *Amb. mexicanum/Tr. cristatus* and control *Amb. mexicanum*. a) *Amb. mexicanum/Tr. cristatus* 346/7. b) *Amb. mexicanum/Tr. cristatus* 438/9. c) Control *Amb. mexicanum* 352/3.

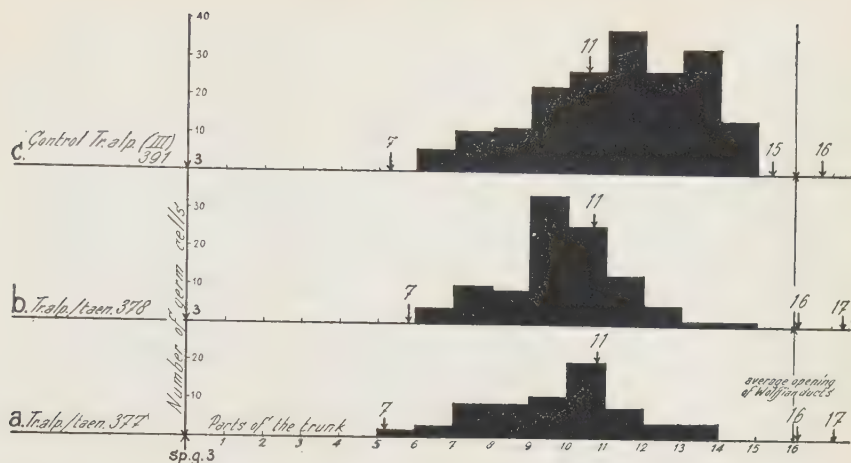


Fig. 52. Id., experimental animals *Tr. alpestris* III/*taeniatus* VIII and control *Tr. alpestris* III. a) *Tr. alpestris*/*taeniatus* 377. b) *Tr. alpestris*/*taeniatus* 378. c) Control *Tr. alpestris* 391.

part of the trunk. In the animals *Tr. alpestris*/*taeniatus* 377 and 378, and *Amb. mexicanum*/*Tr. cristatus* 346/7, the distribution of the germ cells corresponds practically to those of the controls as far as the eleventh spinal ganglion. Then the germ cell number rapidly decreases and becomes zero on about the level of the 14th spinal ganglion. In *Amb. mexicanum*/*Tr. cristatus* 348/9 and *Tr. cristatus*/*alpestris* 356/7, however, most of the germ cells are localized between the 11th and 17th spinal ganglion. A fair number of germ cells are found in the caudal part of the trunk of *Tr. alpestris*/*cristatus* 342/3, while *Tr. alpestris*/*cristatus* 345 even shows a germ cell number and distribution which fairly well correspond to those of the controls.

Discussion.

The distribution of the germ cells in *Tr. alpestris*/*taeniatus* 377 and 378 and *Amb. mexicanum*/*Tr. cristatus* 346/7 may be explained in the same way as in series 11. Here all the germ cells present may have been determined before the operation, whereas the assumption of an induction by the foreign entoderm is unnecessary. This mode of explanation, however, is no longer satisfying when we compare the distribution of the germ cells in the animals *Amb. mexicanum*/*Tr. cristatus* 348/9, *Tr. cristatus*/*alpestris* 356/7 and *Tr. alpestris*/*cristatus* 342/3 and 345, and cor-

responding controls, with that in the entoderm-free *Tr. taeniatus* animals of series 11.

The invagination of the mesoderm in *Tr. alpestris*, *Tr. cristatus* and *Amb. mexicanum* probably corresponds chiefly to that in *Tr. taeniatus*. We may conclude, therefore, that in the last mentioned animals of this series (series 12) the determination of the germ cells has progressed in two phases. In the first the determination of the germ cells in the cranial part of the "germinal region" (as far as the eleventh to twelfth spinal ganglion) has occurred by the entoderm of the host prior to the operation. In the second, the grafted foreign entoderm has also induced the differentiation of germ cells in the posterior part of the trunk.

This experimental series, although furnishing no absolute proof, assuredly supplies a clear indication that the germ cells originate from the mesoderm. This implies, moreover, that the entoderm forms the induction centre, and that its action is indispensable to the differentiation of the presumptive germ cells.

The 4 *alpestris* germ cells in the right germ ridge (3) and in the intestinal epithelium (1) of *Tr. cristatus/alpestris* 356/8 have probably originated from some *alpestris* mesoderm cells which had been grafted by mistake, together with the entoderm into the *cristatus* ecto-mesodermal envelope.

The question remains as to why most of the experimental animals show a smaller number of germ cells than the controls in spite of the presence of entoderm.

At early developmental stages, neurula and early tail-bud stage, the blastoporal lips of these embryos formed a funnel-shaped elevation on the caudal end of the embryo, in which the entoderm was absent. The rounded entoderm had withdrawn from the caudal mesoderm. Nevertheless, a rather normal embryo had been formed at older stages. The larvae, however, still showed a narrow intestinal tube in the posterior part of the trunk. In several cases, it ended more cranially than in normal animals with an irregular slit-shaped anal opening in the ventral median line. We may, therefore, explain the absence of germ cells in the hind part of the trunk by the lack of a topographical contact between the dorso-caudal entoderm and the mesoderm in these segments of the trunk. No determination of germ cells could, therefore, have taken place in these body segments. But in more cranial segments this contact was definitely present.



Fig. 1. *Tr. cristatus/alpestris* 446/7, section 350. Enlargement 225 $\times$. 1 and 2: *Tr. alpestris* and *Tr. cristatus* germ cells respectively. Similarly; 3 and 4: Follicle cells; 5 and 6: Peritoneum cells of somatopleure; 5' and 6': Id. of splanchnopleure; 7 and 8: Mesenchyme cells of somatopleure; 7' and 8': Id. of splanchnopleure; 9 and 10: Smooth muscle cells of splanchnopleure; 11: Amoebocyte in splanchnopleure; 12: Chromatophore; 13 and 14: Striated muscle fibres.

c) Series 13. Heteroplastic transplantation of the presumptive lateral plate and nephrogenous cord mesoderm at the early yolk plug stage.

Experiment and results.

A *Tr. cristatus* and a *Tr. alpestris* embryo, both in the early yolk plug stage, were turned with their blastoporal sides upwards (fig. 53). With a glass needle the latero-ventral mesoderm was extirpated and grafted into the corresponding wound field of the other embryo. The graft was first cut along its animal border then along its dorsal and ventral sides. It was thereupon lifted up carefully and turned back. Adhering entoderm cells were

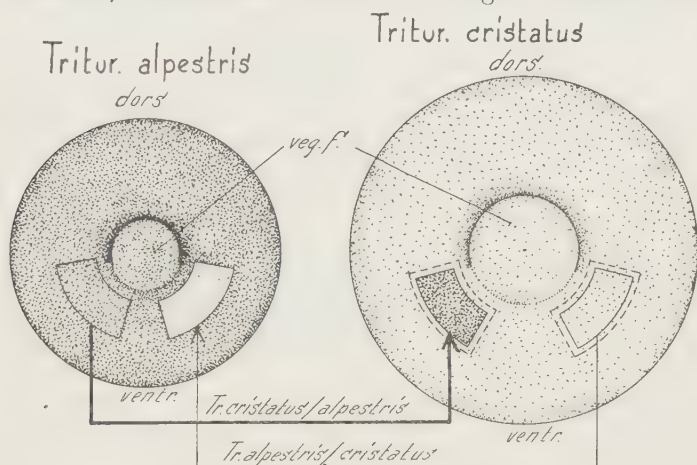


Fig. 53. Operation sketch of heteroplastic transplantation of presumptive lateral plate and nephrogenous cord mesoderm at early yolk plug stage between *Tr. alpestris* and *Tr. cristatus*. Continuous line upon *Tr. alpestris* embryo: Presumable germ cell field. Broken line upon *Tr. cristatus* embryo: Corresponding germ cell field (same relative size). Continuous lines upon *Tr. alpestris* and *Tr. cristatus* embryos: Outline of graft. Enlargement $\pm 15\times$.

removed, and after this the graft was cut along its fourth, vegetative, side. In almost every case, the operation was performed on both sides. ¹⁾

¹⁾ Near the animal border of the ventro-lateral marginal zone, the coherence of the cells was much firmer than near the vegetative field. Here it formed a regular cell layer, on which adhering entoderm cells are easily recognizable. Beginning at this side, this cell layer could be separated without difficulty from the underlying entoderm, and an exact removal of adhering entoderm cells could be readily performed.

A narrow strip of tissue remained between the vegetative border of the graft and the vegetative field. As we have seen above, this material will form the ventral, ventro-cranial and cranial margins of the invaginated lateral plate mesoderm. A narrow band of medio-ventral presumptive mesoderm was also left in place. This would have furnished the caudal parts of the lateral plate and nephrogenous cord. Cf. fig. 35, p. 97.

The transplantation had been performed in the manner described above for two reasons. 1) The exact position of the presumptive lateral plate and nephrogenous cord mesoderm was not yet precisely known at the time of operation; my vital-staining experiments were made a year later. I therefore grafted the latero-ventral and ventro-lateral blastoporal lip only. The vital-staining experiments showed, however, that the medio-ventral blastoporal lip also furnishes parts of the lateral plate and nephrogenous cord mesoderm. 2) In order to prevent as far as possible disturbances in the gastrulation process, I left the cell strip bordering the vegetative field in its place. Notwithstanding this, the *Tr. alpestris* embryos often exhibited considerable disturbances in the gastrulation. The yolk plug was not entirely invaginated and remained visible for a considerable part of the larval period. Not before the consumption of the yolk material did it disappear gradually. Apparently, the gastrulation process in this *Tr. alpestris* egg material was easily disturbed, for the *Tr. cristatus* embryos did not show similar phenomena in the reciprocal transplantation. These disturbances cannot be considered as a kind of exogastrulation, however; only the invagination of the yolk plug and sometimes of the caudal mesoderm has been deranged. The larvae did not show microcephaly or other abnormalities in head or trunk, while the determination of the germ cells took place normally. Only in cases of grave disturbances was the invagination of the ventral to lateral mesoderm also deranged. Only in these animals has the differentiation of germ cells been partly or wholly prevented.

Except for the occurrence of gastrulation disturbances in several *Tr. alpestris* embryos, the grafts soon healed and invaginated normally. The *Tr. cristatus* embryos exhibited an entirely normal development and differentiation; the foreign cells formed one whole with the tissues of the host (cf. Pl. VII). By the quantity and distribution of the pigment the *alpestris* parts of the mesoderm may be distinguished from the *cristatus* tissues in this figure. The larvae were reared for about 25 days after the operation, and were fixed at the stage at which the yolk material had been entirely consumed.

The eggs of *Tr. alpestris* and *Tr. cristatus* are different in diameter. The absolute size of the germ cell fields – as will be proved later, the germ cells originate from the lateral plate mesoderm – will be also different in the two species. The relative size, *i.e.*,

the size in proportion to that of the whole egg, will, however, probably be equal. In consequence of the different absolute size of the germ cell fields in the two species, the *alpestris* graft-graft and wound field were made of the same size - will occupy a larger part of the germ cell field in the *alpestris* than in the *cristatus* embryo. In other words, the *alpestris* graft is relatively larger than the *cristatus* wound field. It will, therefore, contain relatively more germ cells than the extirpated cell material in the *cristatus* host. In the *Tr. alpestris/cristatus* series inverse proportions may be expected.

It will be of importance to compare the proportions of *alpestris* and *cristatus* tissue in lateral plate, nephrogenous cord, germ ridge and entoderm of the various parts of the trunk, with the ratios of *alpestris* and *cristatus* germ cells. I have therefore divided the trunk into a number of parts, each comprising 20 sections of $8\ \mu$ (cf. the continuous lines in fig. 54).

Lateral plate. From the middle section of each of these fillets (cf. the dotted lines in fig. 54), the position of the somatopleure and splanchnopleure has been carefully redrawn with the aid of a ZEISS drawing glass. With an oil

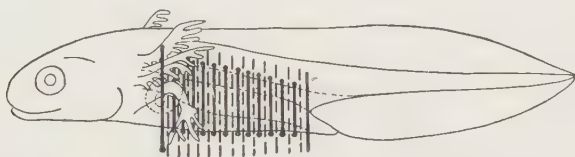


Fig. 54. *Triturus* larva, stage 42 (GLAESNER), left lateral view. Trunk (from forelimb to opening of ectodermal proctodaeum) divided into fillets of 20 sections ($8\ \mu$) between continuous lines. Broken lines: Middle section of every fillet. Enlargement $\pm 7\times$.

immersion, the extent of the *alpestris* and *cristatus* tissue has been determined and inserted in the drawing. Cf. fig. 55.

In the splanchnopleure the boundary between the cell materials of the two species can be determined exactly. In the somatopleure, however, with its scattered cells, muscle plate and often irregular shape, this presents greater difficulty. Moreover, the boundary between the *alpestris* and *cristatus* tissues in the splanchnopleure corresponds for the greater part to that in the somatopleure, and can be determined much more easily and precisely in the former. Therefore the proportion of the tissues in the splanchnopleure of *alpestris* and *cristatus* was compared with the proportion of the corresponding germ cell numbers.

To this end, the length of the *alpestris* and the *cristatus* portions of the left and right splanchnopleures in the enlarged projections of the drawings have been determined with the aid of a curvimeter. Then the proportion percentage between these parts has been calculated. For example, we can



Fig. 55. Heteroplastic transplantation of presumptive lateral plate and nephrogenous cord mesoderm in *Tr. cristatus/alpestris* 460/1. Outlines of somatopleure and splanchnopleure of every 20th section, redrawn with enlargement $\pm 33\times$. Dark line: *Tr. alpestris* cell material, fine line: *Tr. cristatus* cell material, fine dotted line: Median line of splanchnopleure.

compare the drawing in Pl. VII with the vertical line 350 in the diagram in fig. 59 (p. 137).

Nephrogenous cord and germ ridge. An exact determination not being possible, I estimated the proportion of *alpestris* and *cristatus* mesoderm in the mesonephros and germ ridge in the various fillets.

Entoderm. Most of the experimental animals showed cells of the graft in the entodermal epithelium of the intestine. The *alpestris* cells present in the *cristatus* intestinal epithelium, or *vice versa*, may be the source of the foreign germ cells. In that case their number (relative to the total number of entoderm cells) must show a relation to the number of the foreign germ cells (relative to the total number of germ cells).

The *alpestris* intestinal epithelium cells containing much more pigment

than those of *cristatus*, could be readily recognized in the *cristatus* host. In the reverse experiment, the determination of the number of *cristatus* intestinal epithelium cells in the *alpestris* host was impossible. The scarcely pigmented cells are distinct only when they form a coherent group of cells. Scattered unpigmented cells are hardly recognizable as *cristatus* cells, the less so as also a sectioned *alpestris* cell may contain hardly any pigment when the pigment is localized in the next section. The determination of the percentage proportion in the entoderm, therefore, could be performed in the *Tr. cristatus/alpestris* series only.

In every other section the number of *alpestris* intestinal cells was counted, while the total number of entoderm cells was determined in every tenth section. From these values the numbers and the percentage proportions of *alpestris* and *cristatus* intestinal epithelium cells have been determined over fillets of 20 sections.

Germ cells. The numbers of *alpestris* and *cristatus* germ cells have been

TABLE 12

Experimental animals <i>Tr. cristatus/alpestris</i>			Experimental animals <i>Tr. alpestris/cristatus</i>			
Number of animal	Number of <i>cristatus</i> germ cells	Number of <i>alpestris</i> germ cells	Number of animal	Number of <i>alpestris</i> germ cells	Number of <i>cristatus</i> germ cells	<i>Alp.</i> part of lateral plate along germinal region in %
C II 423/4	262	0	443/2	3 (50) ¹⁾	82	23%
457/8	173	0	452/3	2 (63)	116	29%
C III 439/8	343	3	431/2	3 (144)	50	66%
462/3	89	195	429/30	1 (61)	193	28%
448/9	277	44	421/2	0 (?)	10?	abnorma animals
446/7	287	55	450/1	0 (?)	0?	
440/1	344	35				
460/1	59	141	437	2	0	100% „
			420	0	0	100% „
Controls <i>Tr. cristatus</i>			444/5	51	0	100%
C II	258		435/6	47	0	100%
C III a	287		433/4	24	0	100%
b	288		455/6	39	0	100%
c	324		427/8	123	0	100%
C III 3 × M = 300			425/6	183	0	100%
			459/4	184	0	100%
Controls <i>Tr. alpestris</i>						
			II B a	218		
			b	217		
			c	218		
			3 × M = 218			

¹⁾ calculated *alpestris* germ cell number.

determined in ten alternating sections of a fillet (between two successive continuous lines in fig. 54).

The proportion of the numbers of *alpestris* and *cristatus* germ cells can only be compared with the proportions of *alpestris* and *cristatus* material in the other tissues, when the total numbers of germ cells of the controls of the two species are equal. This not being the case, a correction has to be made. Table 12 shows that the total numbers of germ cells of the *Tr. alpestris* and *Tr. cristatus* controls stand in the proportion of 218:300. Therefore I multiplied the number of *alpestris* germ cells by 100/73, and then determined the percentage proportion of the *alpestris* and *cristatus* germ cell numbers in every fillet of 20 sections. The values obtained in these calculations have been expressed diagrammatically.

Since it was often difficult to determine the exact position of graft and wound field at the early yolk plug stage, the position of the grafted mesoderm in donor and host sometimes deviated from the desired position. The microscopic analysis of the experimental animals shows that in some cases the graft has been displaced (displaced wound field), so that the position of the grafted mesoderm in the various hosts differs rather considerably. Therefore some animals will be described separately.

Table 12 shows the numbers of *alpestris* and *cristatus* germ cells of all the experimental animals.

Description of all the experimental animals of series *Tr. cristatus/alpestris*.

In some animals the graft has been displaced dorsal- and cranialwards, so that it partly occupies the pronephros rudiment. The outgrowing Wolffian duct, consisting of graft cells, passes the germinal region; no germ cells, however, differentiate from its tissue. For instance, in *Tr. cristatus/alpestris* 423/4 (1) the grafts are entirely localized before the germinal region. Within this region only the right Wolffian duct consists of *alpestris* cell material for almost its entire length. The germ cells have exclusively an *cristatus* (host) character. In *Tr. cristatus/alpestris* 457/8 (2) the grafts furnish only myotome tissue.

In *Tr. cristatus/alpestris* 439/8 (3) the left graft has taken a more dorsal and cranial position in the host than it had in the donor. Anterior to the germinal region it forms the ventral part of the myotomes and the pronephros. The left Wolffian duct, grown out from the left pronephros rudiment, has an *alpestris* character up to its end in the ectodermal protodaeum. No germ cells have been differentiated from this graft. On the right side, other relations

are found. The right nephrogenous cord (mesonephros) exhibits an exclusively graft character over its whole length; also the germ ridge, *viz.*, from its fore-end to section 450 — *i.e.* nearly the whole germ ridge. Then it contains both *alpestris* and *cristatus* cells as far as section 470, whereas its most caudal part consists of *cristatus* tissue only. Notwithstanding this graft tissue is present along the germinal region of the host, nearly all the germ cells have a *cristatus* (host) character. Only 3 *alpestris* germ cells are present.

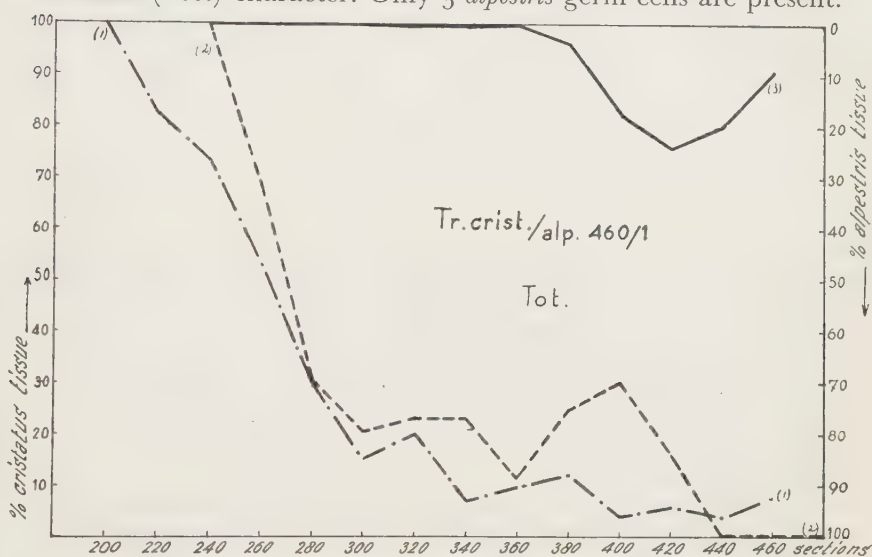


Fig. 56. Heteroplastic transplantation of presumptive lateral plate and nephrogenous cord mesoderm in *Tr. cristatus/alpestris* 460/1. Diagrams of proportions between *Tr. alpestris* and *Tr. cristatus* cell material in lateral plate (— · — · — 1), germ cells (— — — — 2) and entoderm (————— 3). Horizontal: Number of section. Vertical: % proportion *Tr. alpestris* and *Tr. cristatus* cell material. Diagram Tot.: Right and left flanks together.

Tr. cristatus/alpestris 460/1 (8) (fig. 55 and 56) is highly interesting. The lateral plate consists for the greater part of *cristatus* mesoderm in the anterior portion of the germinal region, whereas in its middle and caudal parts the *alpestris* cell material predominates. The germ cell curve follows the lateral plate curve over its entire length. In the cranial portion of the germinal region, the germ cells have chiefly a *cristatus* character, whereas in the middle and caudal trunk region they consist for the greater part of *alpestris* cells.

Only in the posterior part of the trunk is there a top of *alpestris* intestinal epithelium cells. They probably consist of cell material

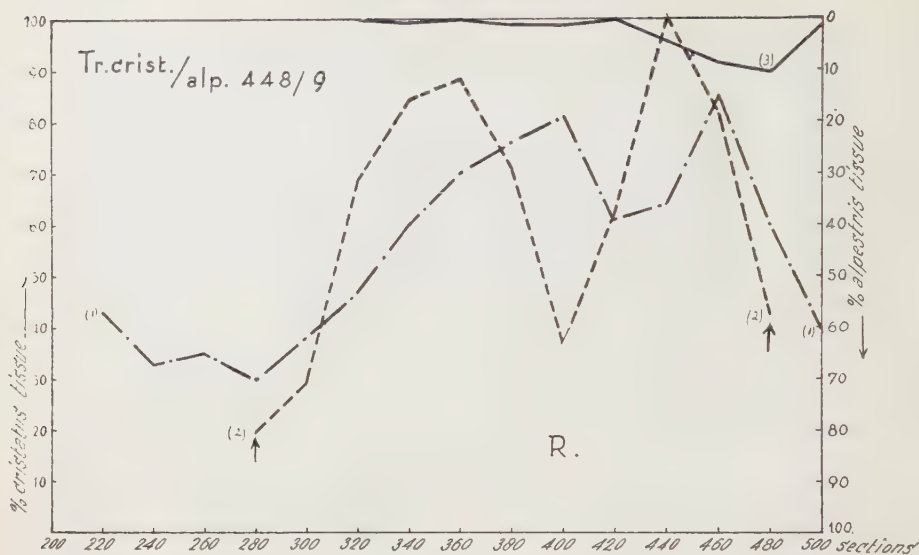


Fig. 57. Id., *Tr. cristatus/alpestris* 448/9. Diagram R. Explanation cf. fig. 56.

of the ectodermal proctodaeum that has been assimilated into the intestinal epithelium (cf. p. 140 (note 1)). No correlation exists between the proportion of graft and host intestinal epithelium cells and germ cells.

In *Tr. cristatus/alpestris* 448/9 (5) all the germ cells of the left side and the whole lateral plate of this flank have *cristatus* character, only a few graft cells are present in the intestinal epithelium. On the right side (fig. 57), however, the lateral plate consists of *alpestris* and *cristatus* tissue in varying proportions along the germinal region. The proportion between the number of *alpestris* and *cristatus* germ cells follows this course roughly.¹⁾

In fig. 58 of *Tr. cristatus/alpestris* 462/3 (4), in the cranial part of the germinal region the lateral plate consists of graft tissue only, whereas the intestine contains hardly any *alpestris* cells. The germ cells have exclusively an *alpestris* character. In the middle part of the germinal region the graft decreases in size, so that about 1/3 of the lateral plate consists of *cristatus* cell material. This part of the trunk contains *alpestris* and *cristatus* germ cells cor-

¹⁾ The presence of graft germ cells on one side only demonstrates that the germ cells of the right and left sides do not pass the median plane, but migrate separately to the corresponding germ ridges. The same phenomenon is seen in *Tr. cristatus/alpestris* 439/8.

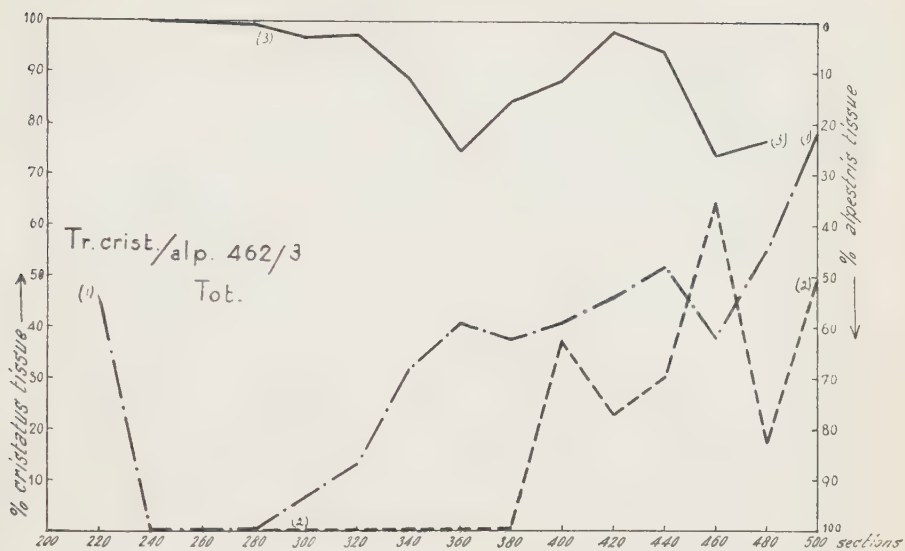


Fig. 58. Id., *Tr. cristatus/alpestris* 462/3. Diagram Tot. Explanation cf. fig. 56.

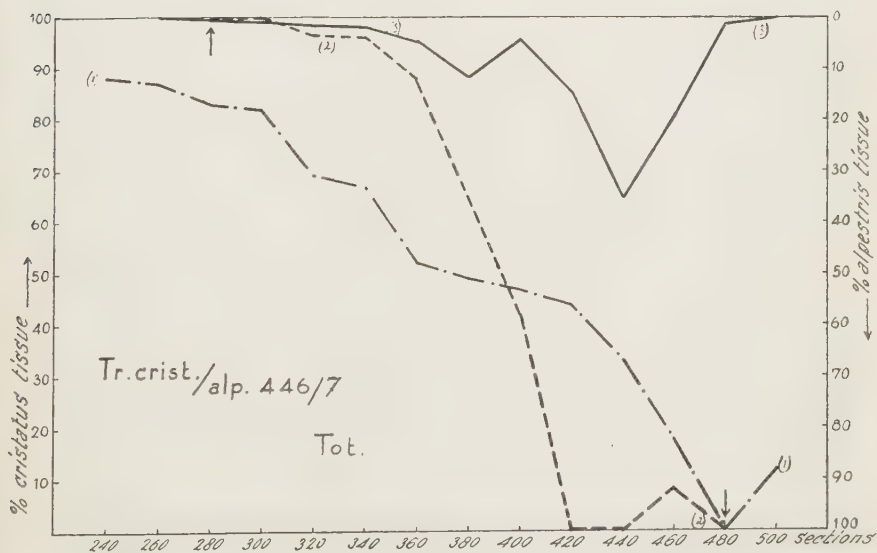


Fig. 59. Id., *Tr. cristatus/alpestris* 446/7. Diagram Tot. Explanation cf. fig. 56.

responding in numbers to the proportions in the lateral plate. The *alpestris* tissue in the intestinal epithelium, however, shows irregular values, and maximally occupies $1/4$ of the cross section of the intestine. In the posterior part of the trunk, the *alpestris* lateral plate and germ cell values decrease still more.

In *Tr. cristatus/alpestris* 446/7 (6) (fig. 59) the lateral plate consists almost entirely of *cristatus* (host) tissue in the anterior part of the germinal region. On the contrary, in the posterior part the proportion between *alpestris* and *cristatus* tissue has changed in favour of the graft. Parallel to this course of the lateral plate proportions, the germ cells have exclusively a *cristatus* character in the cranial part and an *alpestris* character in the caudal part of the germinal region. In the middle trunk region intermediate proportions exist in lateral plate and germ cells. The line of the graft cells in the intestinal epithelium shows one top in the posterior part of the trunk, and occupies maximally $1/3$ of the cross section of the intestine. At this level all the germ cells have an *alpestris* character, however.

Finally in *Tr. cristatus/alpestris* 440/1 (7) the proportions of lateral plate and entoderm are rather irregular. I can merely say that the germ cell proportions correspond better to the lateral plate than to the entoderm proportions.

In *Tr. alpestris/cristatus* 444/5, 435/6, 433/4, 459/4, 455/6, 427/8 and 425/6, the grafts have a more cranial or dorsal position in the *alpestris* hosts than they would have had in normal development in the donors. In all these animals, the lateral plate consists entirely of *alpestris* (host) mesoderm along the germinal region, and all the germ cells have an *alpestris* character.

The animals *Tr. alpestris/cristatus* 437, 420, 421/2 and 450/1 are very abnormal in structure, due to disturbances in the gastrulation process; they have been eliminated from further investigation. In the other animals of the transplantation series *Tr. alpestris/cristatus*, the number of *alpestris* germ cells is very small; therefore the determination of the percentage proportions of *alpestris* and *cristatus* germ cells has no real importance in these cases. I have therefore used another method.

Similar to the germ cell distribution curves of preceding experimental series, the whole trunk (from the third spinal ganglion to the opening of the Wolffian ducts) has been divided into 16 equal parts. In every part the number of *cristatus* germ cells and the proportions of graft and host tissue

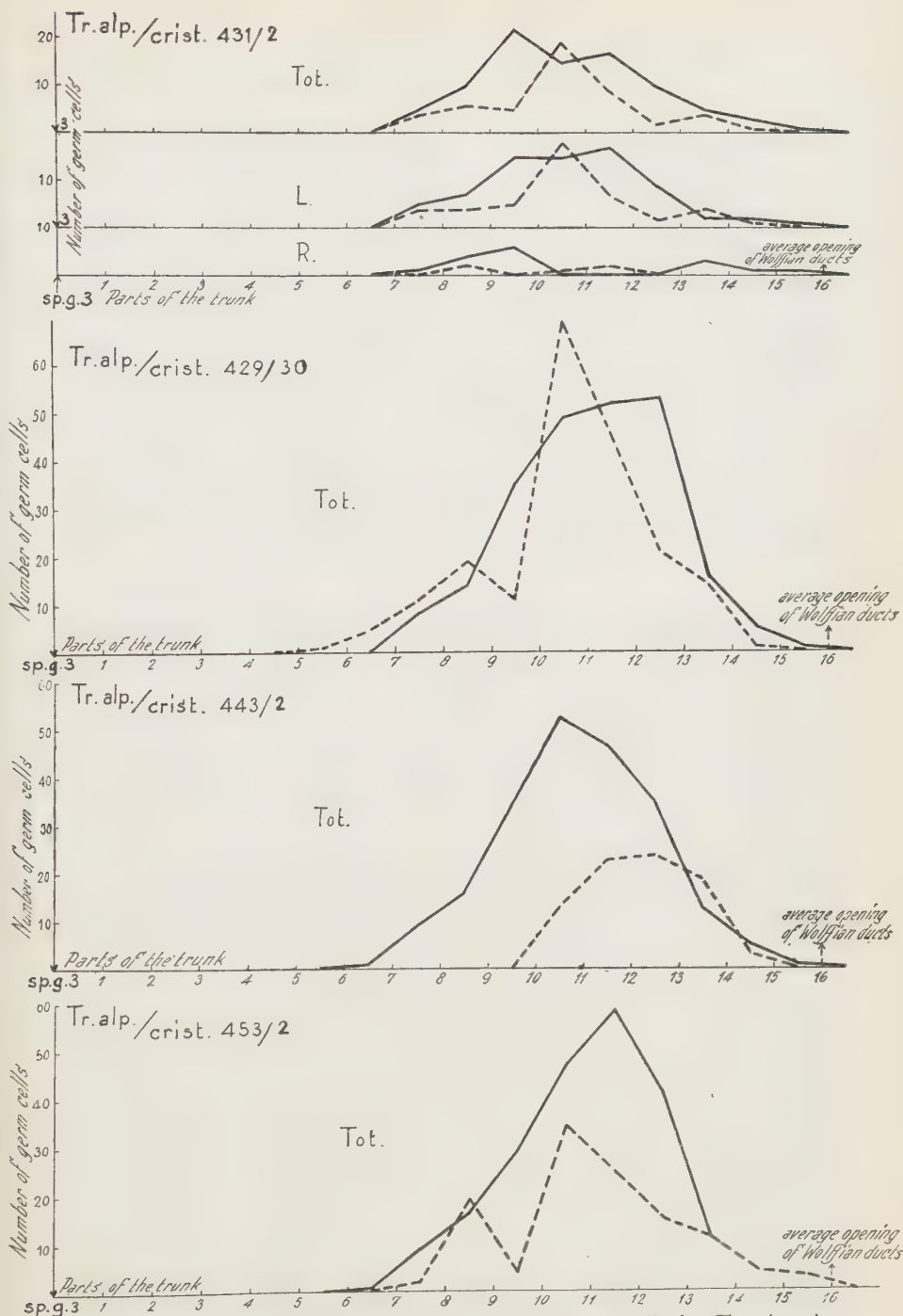


Fig. 60. Diagrams of distribution of *cristatus* germ cells in *Tr. alpestris*. *cristatus* series. Horizontal and vertical cf. fig. 49, p. 125. Broken line: Germ cell distribution observed. Continuous line: Theoretical germ cell distribution calculated from average germ cell distribution curve of *Tr. cristatus* controls, taking into account the % *Tr. cristatus* cell material in lateral plate. a) *Tr. alpestris/cristatus* 431/2. Diagrams R, L and Tot. b) *Tr. alpestris/cristatus* 429/30. c) *Tr. alpestris/cristatus* 443/2 and d) *Tr. alpestris/cristatus* 453/2. Diagrams Tot.

in the lateral plate have been determined. Then, from an average germ cell distribution curve of the *cristatus* controls I have calculated the number of *cristatus* germ cells to be expected in every part separately, taking into account the lateral plate proportions determined.

Except for slight irregularities, the diagrams of *Tr. alpestris/cristatus* 431/2 and 429/30 (fig. 60 a and b) show a great agreement between the *cristatus* germ cell curves of the experimental animals and the theoretical curves. In the first-mentioned animal, where the graft tissue occupies a much larger part of the lateral plate in the left than in the right flank, the foreign germ cells are chiefly localized in the left germ ridge. In the other two animals the lines diverge a little more (fig. 60 c and d). The number is too low in the middle part of the germinal region in *Tr. alpestris/cristatus* 452/3 and in the cranial part in *Tr. alpestris/cristatus* 443/2, where germ cells are even entirely lacking.

Discussion.

The foreign germ cells may have originated from several tissues in these heteroplastic experiments; viz., from graft cells in the intestinal epithelium¹⁾, grafted cell material of myotomes, Wolffian ducts, mesonephric blastemes, germ ridges, or, finally, from grafted lateral plate tissue.

In any case, we must expect therefore a direct relation in the proportions between the number of host and graft germ cells²⁾

¹⁾ The fact that graft cells are present in the intestinal epithelium is very remarkable. These cells may have originated from several sources. The possibility that they have come from grafted entoderm cells may be precluded, as I carefully removed all the adhering entoderm cells during operation. Thus only a mesodermal or an ectodermal origin remains. An ectodermal origin can be imagined only from the ectodermal proctodaeum invagination and may therefore, be expected only in the posterior part of the trunk (cf. figs. 56 and 57). In the middle trunk region, only a mesodermal origin has to be considered. The graft cells are chiefly found in parts of the intestine, adjoining foreign lateral plate tissue. This fact indicates that there must be a relation between the foreign lateral plate tissue and the graft cells in the intestine. Whether we have actually to do with an entodermal differentiation of mesoderm or ectoderm cells, is difficult to decide. I can record only that the graft cells are entirely assimilated into the intestinal epithelium and are similar in appearance.

²⁾ Irregularities in the course of the curves, representing the percentages of *alpestris* and *cristatus* germ cells in the successive parts, may be easily caused. This applies especially to the cranial and caudal parts of the germinal region, where the absolute number of germ cells per fillet is small. The absence or presence of one or two cells may already cause irregularities, as this may even lead to great differences in the percentage proportion.

and the amounts of host and graft cells of the tissue in question.

The proportions between host and graft germ cells and intestinal epithelium cells are shown in the figs. 56-59. I shall consider the various experimental animals successively. From the results obtained in the first animal, *Tr. cristatus/alpestris* 439/8 no conclusions can be drawn; the number of foreign germ cells, as well as the quantity of foreign intestinal epithelium cells, being very small along the germinal region. Fig. 56 of *Tr. cristatus/alpestris* 460/1 demonstrates, however, that the germ cell proportion shows no relation at all to the proportion between host and graft intestinal epithelium cells. In the anterior part of the germinal region, the germ cells have still exclusively a host character, and foreign intestinal epithelium cells are absent. In the middle and posterior part of the germinal region, on the contrary nearly 80% of the germ cells are of graft origin, notwithstanding there are hardly any, foreign intestinal epithelium cells. *Tr. cristatus/alpestris* 448/9 shows that the foreign intestinal epithelium cells are chiefly found in that flank where the lateral plate consists partially of graft tissue. Here again, the small inclusion of graft cells in the intestine does not correspond to the position and the percentage number of foreign germ cells. The following experimental animals *Tr. cristatus/alpestris* 446/7, 462/3 and 440/1 also demonstrate this fact, especially no. 462/3 (fig. 58). Here the germ cells have exclusively a graft character in the anterior part of the germinal region, and foreign intestinal epithelium cells are hardly present. From these experimental animals we may conclude that there is no relation between the percentages of host and graft germ cells and intestinal epithelium cells. In other words, the foreign germ cells have not originated from the graft cells in the intestinal epithelium. This implies that they must have had a mesodermal origin.

In several animals, for instance *Tr. alpestris/cristatus* 459/4 and *Tr. cristatus/alpestris* 439/8 (left flank), the graft has a more dorsal position, and forms myotome tissue. These animals, where only parts of myotomes, situated along the germinal region, have a graft character, contain exclusively germ cells of the host, even in normal number. They prove, therefore, that the foreign germ cells do not originate from foreign myotome tissue.

This corresponds entirely to the defect experiments of the mesoderm, in which the presumptive somites of the posterior

trunk region have been extirpated at the early neurula stage. In the experimental animals a normal number of germ cells was present, from which fact it was evident that the presumptive somite mesoderm has no rôle in the formation of the germ cells.

With respect to the possibility that the Wolffian ducts form the source (or a source) of germ cells, the experimental animals *Tr. cristatus/alpestris* 423/4 and 439/8 (left side) afford a decisive answer. Although in these animals the whole Wolffian duct, grown out from the graft, consists of foreign cell material, all the germ cells have the species characters of the host. Not one foreign germ cell has been formed, whereas a normal number of host germ cells is present. Thus we may also eliminate the Wolffian duct as a source of the foreign germ cells.

A formation of germ cells from foreign mesonephric blastemes or germ ridge cells can also be precluded. As *Tr. cristatus/alpestris* 439/8 (right side) shows, for instance, the whole right mesonephros and nearly the whole right germ ridge (except its posterior end) consist of graft tissue. On the contrary, nearly all the germ cells present in a normal number have the species characters of the host. (The origin of the few foreign germ cells present will be explained below.) The only possibility remaining is that the foreign germ cells have originated from the lateral plate tissue formed by the graft, as ectoderm of the trunk and notochord tissue – the only other tissues not considered above – never contain graft cells. Besides this indirect proof, the figures 56-59 enable us to verify the correctness of this conclusion.

In *Tr. cristatus/alpestris* 439/8, the left lateral plate tissue has a host character along the entire germinal region, and all the germ cells likewise. In the right flank, only a small part of the lateral plate consists of graft tissue. In accordance with this fact, only a few foreign germ cells are present. In comparison with the lateral plate proportions, their number is somewhat too small.

Fig. 56, p. 135, of *Tr. cristatus/alpestris* 460/1 shows an excellent parallelism in the curves of the proportion of host and graft lateral plate tissue and germ cell numbers. In the anterior part of the germinal region, the lateral plate is almost entirely formed by host tissue, while no foreign germ cells are present. Caudad the

percentage of the graft tissue increases considerably in the lateral plate tissue, while, likewise, the proportion of host and graft germ cells changes similarly.

The left lateral plate of *Tr. cristatus/alpestris* 448/9 consists solely of host tissue. Foreign germ cells are entirely lacking on this side of the trunk, whereas a normal number of host germ cells fills the left germ ridge. On the right side, fig. 57, p. 136, the line of the proportions in the lateral plate is rather irregular. The germ cell line roughly follows this latter.

In the following figures 58 and 59, p. 137, especially in fig. 59, there is great agreement between the germ cell and the lateral plate line. In the anterior part of the germinal region of *Tr. cristatus/alpestris* 446/7, the lateral plate is formed for the greater part by host tissue, whereas in the posterior part of the trunk an inverse proportion exists. Parallel to this course of the lateral plate line, the germ cells of the cranial part of the germinal region have exclusively the species characters of the host. In the caudal part of the trunk, however, they belong almost entirely to the graft tissue.

Transplantation series *Tr. alpestris/cristatus*.

Except for slight irregularities the two lines agree very well in *Tr. alpestris/cristatus* 431/2 and 429/30, fig. 60a and b, p. 139. In the first animal, where the graft tissue occupies a much larger part of the lateral plate in the left flank than in the right, the foreign germ cells are chiefly localized in the left germ ridge. In the animals, *Tr. alpestris/cristatus* 443/2 and 453/2 greater irregularities are found, however.¹⁾

¹⁾ In the determination of the theoretical curves in the *Tr. alpestris/cristatus* series, I have assumed that pieces of equal size of both species are comparable. We must, however, call attention to the above-mentioned difference in size of the eggs of the two species. Therefore we have to suppose that a distinct part of the presumptive lateral plate of the *Tr. cristatus* donor occupies a much larger area of the lateral plate in the *Tr. alpestris* host. This implies that the theoretical curves of the germ cell distribution are too high. The differences between the theoretical curves and the curves determined must be considerably reduced in reality.

The sterile anterior part of the germinal region in *Tr. alpestris/cristatus* 443/2 may be explained as follows: During operation, the graft, consisting among other things of the cell material bordering the vegetative field (zone 1 fig. 35a, p. 97), has been grafted at some distance from the blastoporal margin, a fact I had observed. The lateral plate in the anterior part of the germinal region was therefore replaced by more cranial mesoderm,

Our comparison of the quantities of graft and host cells in the various tissues of our experimental animals admits of only one conclusion: The foreign germ cells have actually originated from the foreign lateral plate tissue.

The localization of the presumptive germ cells in the lateral plate may be determined even more exactly yet. In *Tr. alpestris/cristatus* 443/2 the graft, containing the presumptive cell material of more cranial parts of the lateral plate, has been implanted farther from the blastoporal margin than its original position. The anterior part of the germinal region is sterile in this animal. This indicates that the presumptive mesoderm of the anterior part of the lateral plate (before the germinal region) does not contain presumptive germ cells. Therefore we may suppose that the presumptive germ cells are situated in that part of the presumptive lateral plate mesoderm only which will form the lateral plate in the germinal region. In other words, the region with presumptive germ cells normally coincides with the fertile and with the germinal region.

In the various experimental animals in which always other parts of the lateral plate are found to consist of graft tissue, the number of foreign germ cells corresponds rather well to the lateral plate proportions. This would not agree with a localized position of the germ cells in the presumptive lateral plate mesoderm. We may therefore conclude that the presumptive germ cells are scattered among the whole presumptive lateral plate mesoderm of the germinal region. The question as to whether the presumptive germ cells are situated in the presumptive somatopleure or splanchnopleure is of no importance, as the separation of the two layers begins at a stage when the determination, and probably the migration, of the germ cells too, has already taken place.

This series gives additional support to my opinion that secondary germ cells are not formed, at least, not prior to the stage of fixation. Cf. p. 113. For instance, in *Tr. cristatus/alpestris* 439/8 the right germ ridge has exclusively a graft character, whereas in

which evidently contained no presumptive germ cells.

The *cristatus* germ cell curves (observed) as a rule agree rather well with the theoretical curves in the *Tr. alpestris/cristatus* series. So we may conclude that the extremely aberrant proportions of host and graft germ cells in this series has entirely to be accounted for by a considerable deficiency in the number of *alpestris* germ cells.

almost the whole germ ridge only host germ cells are present. An induction of sec. p. germ cells by the p.p. germ cells is, therefore, very improbable. The same holds good for an origin of the sec. p. germ cells from the mesonephric blastemes, myotomes, Wolffian ducts, intestinal epithelium cells, etc. From this series and series 10a we may conclude, therefore, that it is very improbable that sec. p. germ cells are formed during the early larval development (up to stage 42/43 GLAESNER).

A remarkable fact of these transplantation series is the position of the graft in the various experimental animals. In the *Tr. cristatus/alpestris* series the transplant nearly always occupies a part of the lateral plate. In the inverse transplantation, however, it is to be found in many cases in the somites dorsally to the nephrogenous cord mesoderm. In all these cases no germ cells have been formed by it. In some animals this may be caused by a wrong position of the graft in the host, but in many cases it has probably to do with a retardation in the invagination. In many experimental animals of the *Tr. alpestris/cristatus* series, disturbances in the gastrulation have been observed. They represent in reality a retardation in the invagination of the yolk plug and probably of the marginal zone. In consequence of this phenomenon the graft remained for a longer period in the neighbourhood of the blastopore, whereby the dorsal convergence proceeded longer.

The absence of the differentiation of germ cells from grafts situated in an abnormal position – the normal position is the lateral plate within the germinal region – allows of certain conclusions; viz., that the germ cells can differentiate in the presumptive lateral plate mesoderm only within the germinal region, at least if this mesoderm has really formed lateral plate tissue.

In controls germ cells are never formed in the lateral plate mesoderm anterior to a certain level in the trunk. Also experimental animals, in which the graft forms lateral plate tissue in more cranial segments, never contain germ cells before this same level. It is probable, therefore, that a distinct region exists in the caudal half of the trunk in which the germ cells can differentiate. I have termed this the germinal region. The position of this germinal region is probably determined by regional inductive influences in the mesoderm along the longitudinal axis of the trunk.

As in the experimental series no. 10a, there are indications in

this experimental series also, especially in the *Tr. alpestris/cristatus* animals, that a regulation of lateral plate tissue from adjacent mesoderm has taken place, without differentiation of germ cells. In several *Tr. alpestris/cristatus* animals we find, namely, a considerable deficiency of *alpestris* germ cells (cf. table 12, p. 133), whereas the number of foreign germ cells corresponds to the quantity of graft lateral plate tissue. This indicates that the remaining portion of this presumptive lateral plate mesoderm of the host has been supplied by adjacent mesoderm.

The failure of germ cells to appear in regulated lateral plate mesoderm (regulated from other mesoderm) points to the probability that the presumptive germ cells are localized in the presumptive lateral plate mesoderm only. Moreover, it indicates that they cannot differentiate from common lateral plate cells. The experimental series no. 10a may lead to objections being raised to this supposition, *viz.*, the regulation in the mesoderm may have taken place at too late a developmental stage, when the graft cells have already been determined definitely. Moreover, the induction of the dorso-caudal entoderm may have been already considerably weakened. These objections, however, do not hold in this experimental series, as the operation has been performed at the early yolk plug stage. At that stage a considerable regulation quickly begins after injury to the marginal zone. These heteroplastic transplantation experiments, therefore, support the assumption of a predetermined state of the presumptive germ cells in the presumptive lateral plate mesoderm. An infallible proof of this conception, however, has still to be furnished.

E. BRIEF DISCUSSION OF VARIOUS PROBLEMS CONCERNING THE ORIGIN OF THE GERM CELLS.

(Supplement to p. 111-114, 117-119 and 140-146.)

I never found in my experimental animals any indication of the formation of a second generation of germ cells. After the removal of the presumptive germ cell material, no regeneration of germ cells from the germ ridges takes place. In the case of an almost complete removal, the experimental animals possess but few germ cells, as is to be expected. In the heteroplastic transplantations, the germ ridges contain numbers of germ cells of the two species, proportionate to the ratio of host and graft cells in the lateral plate, not to that in the germ ridges. The whole, or

part, of the germ ridge may even contain exclusively germ cells of the other species, which proves that the possibility of an induction of sec. p. germ cells by the p.p. germ cells may be precluded too.

The assumption of a second generation of germ cells, originating from differentiated somatic cells, based upon arguments of KUSCHAKEWITSCH, BECCARI and others (cf. p. 43), was criticized by WITSCHI (1914). Neither have I ever observed any considerable degeneration of germ cells, even in my experimental animals. I found some degenerating germ cells in a few cases only among the large number of experimental and control animals examined. In my experiments not only the number of germ cells, but also the differentiation or, in other words, the number of characteristics of the germ cells decreased after the removal of the dorso-caudal entoderm. This proves that the differentiation of the germ cells is dependent upon the intensity of the inductive action. The "badly" differentiated germ cells bear a great resemblance to so-called "transitional stages between somatic cells and germ cells", but in reality their significance is quite another. From these facts we may conclude that during the development of the larva, at least up to stage 43 (GLAESNER), no second generation of germ cells will probably appear.

As I mentioned already, most of the facts plead in favour of an entodermal origin of the germ cells in the Anures, although the experiments of MONROY and BOUNOURE are not wholly convincing in this respect. Their results may also be explained by a removal, or destruction, of the inductive centre, so that the indispensable determination is lacking. On the contrary, in the Urodeles the experimental facts prove a mesodermal origin of the p.p. germ cells. Considering the large individual variations in the number of germ cells, the contrary result arrived at by SEMBRAT loses its value.

My experiments have proved that the germ cells originate from the presumptive lateral plate mesoderm, that is to say, from the ventral and latero-ventral blastoporal lips of the gastrula. HUMPHREY's hypothesis (1925) of an origin of the germ cells in the Urodeles from the blastoporal lips has been confirmed by the facts. The exact position of the presumptive germ cells could not have been foreseen by him, however.

Comparing the origins of the germ cells in the Anures and the Urodeles, we may state that an early segregation of the germ cells occurs in both groups, but, on the other hand, a wide gap appears in the place of origin of the germ cells. We can project the position of the presumptive germ cell material back upon the fertilized egg. In the Urodeles, it will correspond to the plasma situated at the equator of the egg, diametrically opposite the grey crescent. In the Anures, however, the "cytoplasme germinale" is situated at the vegetative pole of the egg. Nothing but an experimental investigation in the Anures will, in my opinion, solve the question, as to whether this wide gap does really exist, or not.

NUSSBAUM (1880), BOUNOURE (1925) and others, hold the opinion that an entodermal origin corresponds to an early segregation of the germ cells from omnipotent embryonic cells. In their opinion a mesodermal origin means the development of germ cells from differentiated somatic cells. The explantation experiments of HOLTFRETER (1938) and the transplantation experiments of MANGOLD (1923), HOLTFRETER (1931a and b, and 1938), BAUTZMANN (1928 and 1933) and others, prove that this view is quite erroneous. According to these writers, the entodermal cell material contains relatively strong differentiation tendencies already at the gastrula stage. On the other hand, the differentiation tendencies are very weak in the mesoderm at this stage. This corresponds to the fact that the mesoderm still contains many differentiation potencies – even ectodermal and entodermal differentiations of mesoderm are known. So the embryonic character (large quantity of yolk) of the entoderm does not point to any omnipotence of this cell material. Moreover, there are morphological indications that the germ cells do not belong to any of the germ layers and thus also not to the entoderm, but represent specific elements (BOUNOURE 1939). My own experiments point in the same direction.

The differentiation of the germ cells in a distinct part of the trunk seems safeguarded by triple insurance. 1) The presumptive germ cells are localized only in that mesoderm of the marginal zone which will form the lateral plate tissue in the "germinal region" of the trunk. 2) Only from the caudal entoderm does an inductive action emanate and act upon the adjacent mesoderm. In other words, the germ cell determination occurs in the caudal

part of the trunk (the "germinal region") only. Cf. p. 190. 3) The differentiation of germ cells, even already determined, can take place only within the germinal region, as in more cranial or caudal parts of the trunk it is prevented by foreign regional inductive influences.

PART II, CHAPTER 2. INDUCTION AND DETERMINATION OF THE GERM CELLS IN THE URODELES.

A) DISCUSSION OF THE PRECEDING EXPERIMENTS IN CONNECTION WITH THE DETERMINATION OF THE GERM CELLS.

From the defect experiments of the caudal entoderm and mesoderm and from the heteroplastic mesoderm transplantations we know 1) that only the presumptive lateral plate mesoderm contains the presumptive germ cells, and 2) that an inductive action is exerted by the dorso-caudal entoderm and that the latter determines the differentiation of the germ cells.

In the experimental series no. 11, in which the whole entoderm had been removed at the early neurula stage, I observed great agreement between the size of the invaginated part of the lateral plate mesoderm (to the level of the 11th-12th somite) and the localization of the germ cells. In this series only the anterior portion of the mesoderm (invaginated part) had come into contact with the caudal entoderm before the removal of the latter, cf. fig. 44, p. 118; in the larva only the cranial part of the "germinal region" contains differentiated germ cells. Hence, only in that part of the presumptive lateral plate mesoderm, that temporarily has bordered the dorso-caudal entoderm, have p.p. germ cells differentiated.

Concerning the course of the induction process we can make the following statements: The mesoderm of lateral plate and nephrogenous cord of the anterior seven body segments, which invaginates during the early yolk plug stage, plays no part in the formation of the germ cells; it is situated before the beginning of the germinal region and, evidently, contains no presumptive germ cells (compare *Tr. alpestris/cristatus* 443/2, p. 139). So, the first segment of the germinal region invaginates no sooner than the intermediate yolk plug stage. The determination of the germ cells in the cranial part of the germinal region cannot begin before this stage.

The post-gastrulation of the latero-ventral and ventral meso-

derm occurs during the neurulation (till the neural folds have closed). The caudal end of the germinal region, forming the caudal segments of the trunk, invaginates at an intermediate neurula stage; in the latter phases of the post-gastrulation it is chiefly tail-bud (and probably posterior nephrogenous cord) mesoderm that invaginates.

The animals of series 11 show well differentiated germ cells in the mesoderm of the 10th and 11th segment, invaginated during the appearance of the neural folds a few hours before the removal of the entoderm. From this fact we may conclude that the induction occurs rapidly. The induction period, therefore, probably extends only from the intermediate yolk plug to the late neurula stage.

An important question is whether an inductive action can actually take place between the two parts or, in other words, whether their topographic relations permit of such an action. I will endeavour to answer this question by considering separately the positions of the two parts at successive stages of the induction period.

At the intermediate yolk plug stage the entoderm has already been invaginated almost entirely, only a tiny yolk plug is still visible externally. The presumptive dorso-caudal entoderm borders the ventral to lateral parts of

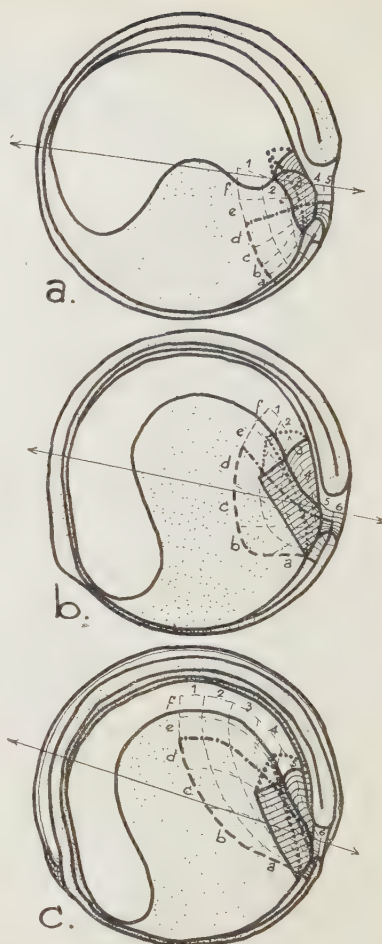


Fig. 61. a) Reconstruction of position of dorso-caudal entoderm (hatched) and presumptive lateral plate and nephrogenous cord mesoderm in right half of embryo at slit-blastopore stage. 4 out of 6 animal-vegetative zones of ventral to lateral mesoderm invaginated. Mesoderm of lateral plate and nephrogenous cord divided into zones a-f in ventro-dorsal direction, cf. fig. 35, p. 97. b) Similar reconstruction at early neurula stage. Beginning of dorsal convergence. c) Id. at middle neurula stage. Strong dorsal convergence. Enlargement $\pm 25\times$.

the mesodermal mantle at this stage. During the later phases of the gastrulation this entoderm is entirely invaginated; the distance over which it is displaced is, however, very small (fig. 61a). At the end of the gastrulation period the lateral borders of the entoderm bottom grow out in a dorsal direction enclosing the gastrocoel; therefore the entoderm borders also more lateral and dorsal parts of the mesoderm. This process, which has begun in the anterior trunk region, proceeds caudad, so that in the posterior trunk region it commences at a later stage. The lateral margins of the most caudal entoderm begin to rise at the early neurula stage, so that the dorso-caudal entoderm now borders also the lateral mesoderm. The ventral entoderm mass is displaced somewhat caudad by the enlargement of the cephalic gastrocoel (cf. p. 68-69). At the same time, the caudal entoderm is for a short distance displaced to the dorsal side (cf. fig. 61b). Finally, at the intermediate neurula stage, the closing of the intestinal roof is completed by the elevation of the lateral edges of the entoderm. Now the dorso-caudal entoderm borders the lateral to dorsal mesoderm. Stretching of the caudal entoderm in a cranial direction has not yet taken place at this stage (fig. 61c).

As regards the mesoderm, I shall confine my description to the displacements of the presumptive lateral plate mesoderm, as the preceding experiments have proved 1) that only the presumptive lateral plate mesoderm forms the source of the germ cells, and 2) that from the neurula the presumptive nephrogenous cord mesoderm is situated dorsally to the presumptive lateral plate mesoderm.

At the early yolk plug stage the presumptive lateral plate mesoderm is situated in the external cell layer ventrally, and latero-ventrally to the vegetative field (fig. 35a, p. 97). At the intermediate yolk plug stage, the lateral plate mesoderm of the anterior part of the germinal region begins to be invaginated round the latero-ventral blastoporal lips. At the slit-blastopore stage the invaginated portion already occupies a triangular field ventrally and latero-ventrally, and even ventro-laterally, to the blastopore (fig. 61a). At the very early neurula stage the shape of the presumptive mesoderm of the lateral plates has become bilobed. It occupies the ventro-lateral and lateral portions of the mesodermal mantle in the caudal part of the embryo (fig. 61b). The dorsal displacement continues during the neurulation. At

the intermediate neurula stage, when the mesoderm of the last part of the germinal region has been invaginated, the presumptive lateral plate mesoderm forms the lateral part of the mesodermal sheet (fig. 61c).

From this description we may conclude that the presumptive lateral plate mesoderm comes into direct contact with the dorso-caudal entoderm during the end phases of gastrulation and during neurulation. The answer to the question as to whether the topographical relations permit an inductive action, therefore, is: An induction can very well take place.

The extirpation and heteroplastic transplantation experiments of the mesoderm indicate that only distinct cells of the original lateral plate mesoderm can be transformed into germ cells. In other words, the presumptive germ cells are present among the presumptive lateral plate cells in a predetermined state. This predetermination means that only such, predetermined, cells have a "reactive power", which enables them to react to special inductive stimuli by the formation of germ cells, whereas this power is lacking in the other cells of the body. The histological investigation of BOUNOURE proves that in the Anures the presumptive germ cells are characterized by the presence of a special "cytoplasme germinal". If this also holds good for the Urodeles, as is not yet known, this would be a strong argument in favour of the above-mentioned predetermination. Moreover, this would furnish an explanation of the fact that only those cells have this "reactive power". In this case, the chain of causal factors leading to the formation of the germ cells would be elucidated to a great extent: A special protoplasmic substance, present in [the uncleaved egg, is segregated into particular cells during cleavage (proved by BOUNOURE in the case of the Anures; the fact remains to be investigated in the Urodeles, however). By this "chemodifferentiation" the cells acquire special properties which distinguish them from other cells; in particular they acquire the "reactive power" enabling them to react to inductive stimuli emerging from the caudal entoderm by the differentiation into germ cells (Urodeles). The question as to whether the presumptive germ cells are already entirely specific elements, or not, will have to be investigated further. Especially the knowledge of the history of the individual germ cells may throw light on this problem.

The fact that the presumptive germ cells cannot differentiate into germ cells if situated in an abnormal position, even after the normal influence from the dorso-caudal entoderm has been exerted upon them, is also of interest. It remains to be investigated whether in that case the presumptive germ cells will merely be prevented from differentiating, or will be transformed into somatic cells by foreign inductive influences. In the transplantation experiments to be described later I have considered the question as to what foreign inductive actions may present the differentiation of the germ cells, and in what manner they act. Finally, the question arises whether the dorso-caudal entoderm is the sole organ which can determine the differentiation of the presumptive germ cells.

B. FURTHER EXPERIMENTS.

Series 14. Removal and transplantation of the pronephros and Wolffian duct rudiment at the early neurula stage.

Experiment and results.

The vital staining and transplantation experiments of O'CONNOR (1938/'39) and of PASTEELS (1942) indicate that the Wolffian duct originates from the caudal part of the pronephros rudiment. According to these authors, it grows out from this point in caudal direction to the cloaca.¹⁾ Other authors hold the opinion that the whole Wolffian duct is formed in situ from the cell material of the nephrogenous cord.

My experiment was carried out in the following way: Cf. fig. 62a. At the early neurula stage an embryo of *Amb. mexicanum* or *Tr. taeniatus* was turned on its right side. The ectoderm was cut cranially, ventrally and caudally to the presumptive (mesodermal) pronephric rudiment,²⁾ carefully separated from the underlying mesoderm, and turned over to the dorsal side. Then the mesoderm was cut along its four sides and separated from the entoderm.

¹⁾ In some cases O'CONNOR observed the development of a small Wolffian duct in the most caudal part of the trunk, communicating with the cloaca, while the normal Wolffian duct had been blocked by a graft in the medial trunk region. From this observation he concluded that the most caudal part of the Wolffian duct is formed from the cloaca.

²⁾ According to YAMADA (1937), the presumptive pronephros rudiment is localized laterally to the neural fold, somewhat behind the middle of the embryo, at the early neurula stage (fig. 62a).

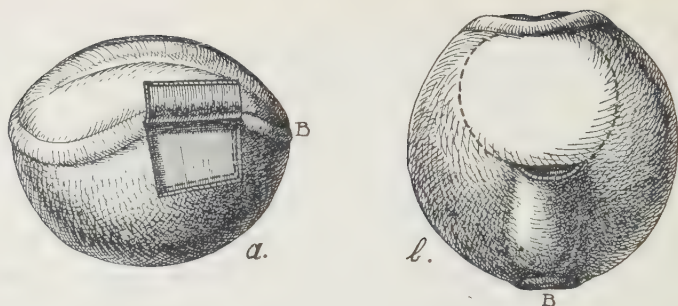


Fig. 62. Sketch of transplantation of presumptive pronephros rudiment at early neurula stage. a) Loosening of rudiment after cutting ectoderm along three sides and folding over. Broken line: Outline of mesodermal graft. b) Formation of pocket-shaped cavity between entoderm and ventral mesoderm, same stage, ventral view. Dotted line: Outline of mesoderm-free area. Enlargement $\pm 14\times$.

After the ectoderm was folded back, the wound soon closed. Like every operation, the experiment must be performed as quickly as possible to facilitate the closing of the wound, and to prevent the curling of the margins of the graft.

The host, an embryo of *Tr. taeniatus* or *Amb. mexicanum* of the same age – only homoplastic transplantations have been carried out – was turned with its ventral side upwards. A small incision was made in the ectoderm cranially to the anterior border of the ventral mesoderm. Then a thin glass rod, terminating in a round globule, was inserted between the entoderm and the medio-ventral mesoderm. In this way a pocket-shaped cavity, communicating through a small opening with the exterior, was made between the two germ layers (fig. 62b). The graft was pushed into this cavity as far as possible in caudal direction (operation-method of YAMADA (1937)). The embryos developed almost normally, and have been reared for 21 days.

In the *Ambystoma* and *Triturus* donors, the pronephros as well as the forelimb rudiment were in most cases almost entirely lacking on the operated side; only a small tubule, or an isolated nephrostome, had been formed sometimes. Cf. table 13b, column 8.¹⁾

¹⁾ In this series I used eggs of two females of *Amb. mexicanum* (A and B). The *Triturus* embryos have not been thoroughly investigated and are not included in the tables.

On the contrary, the Wolffian duct had partly, or wholly, developed in many cases. Only in those animals in which the caudal part of the pronephros rudiment had been completely extirpated, no Wolffian duct grew out. In many animals, the Wolffian duct had developed only partially in the form of small isolated portions, situated at various distances between pronephros and cloaca. In the *Triturus* hosts, the grafts have formed only an accumulation of mesenchymal cells. In the *Ambystoma* hosts they have differentiated into pronephric tubules, blood cells, etc., while in a number of cases a Wolffian duct has also developed.

In order to investigate whether the Wolffian duct influences the determination and distribution of the germ cells along the germinal region, I will divide the donors into three groups. In group I the Wolffian duct is entirely lacking on the operated side, in group II only small parts of it are found along the germinal region, while in group III almost the entire Wolffian duct has been formed on the operated side (table 13b).

TABLE 13a

Controls of series A, *Ambystoma mexicanum*

Number of animal	Number of g. c. left side	Number of extra regional g. c.	Number of g. c. right side	Total number of g. c.	Beginning of fertile region left side	Beginning of fertile region right side
215/6	59	1	51	111	10.5	11.5(10.5) ¹⁾
217/8	98	1	88	187	12.1(11.3)	11.7
219/20	78	1	53	132	11.2	12.—
223/4	50	5	67	122	10.5	10.9
225/6	33	1	72	106	11.3	10.7
227/8	91	1	84	176	9.9 (9.3)	10.7
231/2	64	2	47	113	8.7	9.8
233/4	59	1	78	138	11.5	11.6
235/6	78	2	52	132	9.9	10.7
237/8	60		64	124	10.5	11.6 (9.3)
221/2	42		24	66	13.5	14.4
229/30	17	1	44	62	12.5(11.6)	10.3

$$12 \times \quad M=61 \quad M=1 \quad M=60 \quad M=123 \quad M=11.0 \quad M=11.3$$

$$m=\pm 10.6$$

¹⁾ In cases with double number, first number: Position of actual beginning of fertile region, second number: Position of first germ cell. In average only first value used.

TABLE 13b

Donors of series A, group I, *Ambystoma mexicanum*

Number of animal	Number of g. c. left side	Number of extra regional g. c.	Number of g. c. right side	Total number of g. c.	Beginning of fertile region left side	Beginning of fertile region right side	Defects
XX A	81" ¹⁾		101	182	9.7"	10.1	p.n. f.l. W.d. ²⁾
XVII A	74"	1	71	146	10.9"	11.1	p.n. f.l. W.d.
XIX A	76"	1	57	134	9.5"	10.7	p.n. f.l. W.d.
XVIII A	70"		81	151	12.3"	11.6	p.n. f.l. W.d.

4 × M = 75" M=1 M=77 M=153 M=10.6" M=10.9

Donors of series A, group II, *Ambystoma mexicanum*

XVI A	64"	4	106	174	10.8"	10.4	p.n. f.l. W.d.
XV A	71"		119	190	10.8"	10.4	p.n. f.l. W.d.

2 × M = 68" M=2 M=113 M=182 M=10.8" M=10.4

Total number of g.c., group I and II M=163

Donor of series B, group I, *Ambystoma mexicanum*

XII A	172	5	138"	315	9.3	8.4"	p.n. f.l. W.d.
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Donors of series B, group II, *Ambystoma mexicanum*

VII A	92"	2	144	238	9.1"	8.9	p.n. f.l. W.d.
V A	90"	2	110	202	9.1"	9.7	p.n. f.l. W.d.
XIII A	86"	1	120	207	8.5"	8.6	p.n. f.l. W.d.
IX A	130"	4	165	299	8.1"	8.6	p.n. f.l. W.d.
VI A	133"	1	134	268	8.8"	9.4	p.n. f.l. W.d.
XI A	122"	1	176	299	7.2"	7.4	p.n. f.l. W.d.

6 × M = 109" M=2 M=142 M=252 M=8.5" M=8.8

M (left and right) = 8.6

m = ± 17.7 m = ± 0.23

Donors of series B, group III, *Ambystoma mexicanum*

XIV A	156"	1	123	280	8.9"	8.5	p.n. f.l. W.d. ²⁾
X A	148"	1	141	290	8.4"	9.3	p.n. f.l. W.d.
IV A	185"		186	371	8.9"	8.3	p.n. f.l. W.d.

3 × M = 163" M=1 M=150 M=314 M=8.7" M=8.7

m = ± 28.8

¹⁾ Values, marked with "", are of operated side.²⁾ P.n.: pronephros, f.l.: forelimb, W.d.: Wolffian duct, sp.g.: spinal ganglion, bl.c.: blood cells. Normal symbols signify organ entirely absent, italic symbols organ partially lacking, thick symbols organ almost completely present on operated side.

TABLE 13c
Hosts of series A, *Ambystoma mexicanum*

Number of animal	Number of g.c. left side	Number of extra regional g.c.	Number of g.c. right side	Total number of g.c.	Beginning of fertile region left side	Beginning of fertile region right side	Position of graft with regard to sp. g. ²)	Character of graft
XVIII B	62	5	29	96	12.1	12.5	sp. g. 7-9	p.n. bl.c. ²)
XVII B	126	6	126	258	9.5	10.1	" " 7-10	p.n. fl. bl.c.
XVI B	60		89	149	11.5	10.2	" " 9-12	p.n. fl. bl.c.
XV B	79		53	132	10.3	10.9	" " 5-7	bl.c.
4 ×	M = 82	M = 3	M = 74	M = 179	M = 10.9	M = 10.9		

Hosts of series B, *Ambystoma mexicanum*

XII B	117	5	111	233	9.9	10.9	sp. g. 4-5	p.n. fl. W.d.
XB	103	7	47	157	8.1	10.0	" " 7-10	p.n. 2 fl. W.d.
IV B	53	21	146	220	11.6	9.5	" " 5-6/7	p.n. fl. W.d.
V B	149		191	340	9.4	8.7	" " 7-8/9	p.n. 2 fl. bl.c.
XIV B	86	22	144	252	11.5	9.1	" " 7-10/11	p.n. fl. W.d.
VII B	145	7	110	262	9.5	9.1	" " 5-6/7	p.n. fl. bl.c. W.d.
VIII B	83	79	67	229	9.2	9.2	" " 6-8/9	p.n. fl. bl.c. W.d.
7 ×	M = 105	M = 20	M = 117	M = 242 m = ± 20, 7	M = 9.9	M = 9.5		

Comparing the averages of the numbers of germ cells of donors, hosts and controls, we may conclude that there are no real differences between them. The average number of germ cells of the controls (series A) is somewhat lower than of the corresponding experimental animals. This is partly due to two of the animals containing a very small number of germ cells.

From table 13b we can see that in most of the experimental animals of group I and II the number of germ cells is somewhat lower on the operated than on the non-operated side. In series A the operated side contains on an average 73 germ cells ($m = \pm 2,4$), the unoperated side 89 ($m = \pm 9,6$). In this series $\frac{D}{m_D}$ has the value $\frac{16}{9,8} = 1,6$. In series B on an average 113 germ cells are present on the operated side ($m = \pm 8,6$) and on the unoperated side 146 ($m = \pm 9,8$). Here $\frac{D}{m_D}$ has the value $\frac{33}{13} = 2,5$.

Evidently these differences are not completely conclusive.

The position of the fertile region is but little altered on the operated side. Only in the hosts of series B is the beginning of the fertile region somewhat more caudad than in the donors. The difference between the averages is conclusive, as the average of the host amounts to 9,7 ($m = \pm 0,27$), of the donors 8,6 ($m = \pm 0,23$) and $\frac{D}{m_D} = \frac{1,1}{0,36} = 3,08$.

Discussion.

From tables 13a, b and c we may conclude that neither the total number of germ cells, nor the distribution along the germinal region has been influenced to any extent by the extirpation of the pronephros and Wolffian duct rudiment. The same holds good with transplantation of a supernumerary pronephros rudiment in the medio-ventral mesoderm.

The slight difference in the position of the fore-end of the fertile region, found between donors and hosts in series B, may indicate that owing to the absence of one of the two pronephric rudiments the germinal region extends somewhat more craniad. On the contrary, with the presence of a supernumerary rudiment its fore-end lies somewhat more caudad. This would mean that in all probability the pronephric rudiment also influences the

regional induction of the germinal region (quantitative alteration of gradients?)

Presumably the small differences observed in the number of germ cells on the operated and on the non-operated side in the donors may have been caused by the complete, or partial, absence of any attraction by the Wolffian duct in the first phase of the migration process. (The germ cells are first attracted by the Wolffian ducts and then by the germ ridges. Cf. part III.)

From the positions of the grafts it is evident that, although pushed far caudad beneath the invaginated mesoderm, they have been displaced together with the host mesoderm rather far in a cranial direction. They have been simply enclosed in the surrounding mesoderm and displaced with the normal gastrulation movements.

The differentiation of the graft demonstrates that in *Tr. taeniatus* the determination of the pronephros and Wolffian duct occurs at a later stage than in *Amb. mexicanum*. It proves, moreover, that also in *Ambystoma mexicanum* the pronephros rudiment is not yet completely determined at the early neurula stage, since a complete differentiation has never taken place. The differentiation of blood cells in these but "weakly" determined pronephros rudiments is in complete agreement with the results of YAMADA (1937 and '38).

The differentiation of the pronephros graft into tubules with mesonephros character, as found in some animals, may be connected with the position of the graft in the mesonephros region (a more caudal position than it normally occupies). This supposition, however, has still to be investigated further. Compare MASCHKOWZEFF (1934/'35).

The differentiation of the grafted pronephros rudiment is often not complete; irregular and incomplete pronephros tubules (nephrostomes are always lacking) and blood cells (Cf. DALCQ (1941)) are to be found. In many cases forelimb rudiments have actually been formed from the grafts. From the fact that the donors never show a forelimb rudiment on the operated side, it is evident that the presumptive forelimb rudiment has been transplanted together with the presumptive pronephros rudiment. The differentiation of forelimbs in the grafts indicates that the forelimb rudiment has already been determined at the stage of operation. This agrees with the observation of DETWILER (1929 and '33), that in *Amb. punctatum* the determination of the forelimb begins already at the late yolk plug stage.

As only the pronephros rudiment has been extirpated, the reduced Wolffian ducts in the donors can be explained only by assuming that the Wolffian duct grows out from the caudal part of the pronephros rudiment. Every single cell of the Wolffian duct rudiment must have the power to migrate caudalwards, as I frequently observed isolated groups of cells forming a small but characteristic Wolffian duct. A differentiation in situ cannot explain these facts. In four hosts a supernumerary Wolffian duct, originating from the grafted pronephros, has developed, running in caudal direction through several body segments. In three of these animals it runs in dorso-caudal direction through the coelomic cavity, and communicates with one of the Wolffian ducts of the host. It might still be possible that, during the

displacement of the graft from immediately in front of the blastopore to its final position in the medial trunk region, the pronephros rudiment has left behind cells which have differentiated in situ. This possibility may be rejected, since the Wolffian duct followed a course which does not coincide with the displacement of the graft. Therefore a differentiation in situ is out of question here, so that we are forced to conclude to the existence of an active growth of the Wolffian duct from the caudal part of the pronephros rudiment. In the last-mentioned cases the supernumerary Wolffian duct runs in a dorso-caudal direction, while the Wolffian duct of the host has been displaced to the ventral side. This indicates that there must be mutual attraction between the two ducts (MASCHKOWZEFF (1934/1935)).

A supernumerary germ ridge has been formed along the supernumerary Wolffian duct in three of the four cases. Cf. part III of this publication, p. 180.

Series 15. Transplantation of the notochord at the early neurula stage.

Experiment and results.

Operation method (fig. 63). In an *Amb. mexicanum* embryo two cuts were made in the neural plate from the blastopore along the inner sides of the neural folds, without injuring the underlying mesoderm. Then the neural plate was separated from the chordo-mesodermal plate, from the blastopore to the middle of the neural plate, and lifted craniad. In the underlying chordo-mesodermal plate the caudal half of the notochord – which is bounded by two longitudinal lines – was cut loose from the right and left mesodermal plates. Then it was separated from the underlying dorsal entoderm walls, and finally removed. The neural plate flap was folded back against the mesoderm and neural folds; the edges of

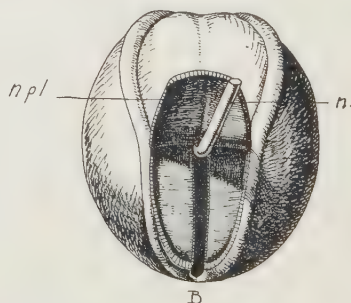


Fig. 63. Diagram of removal of notochord at early neurula stage. Neural plate cut along lateral edges and bent craniad. Notochord isolated from the back forwards. Enlargement $\pm 14\times$.

the wound soon grew together. In order to prevent the curling of the margins of the neural plate, a glass bridge was often used. In the hosts a sac-like cavity was made beneath the medio-ventral mesoderm – as in the pronephros transplantation experiments – and the long graft was pushed into the cavity. In

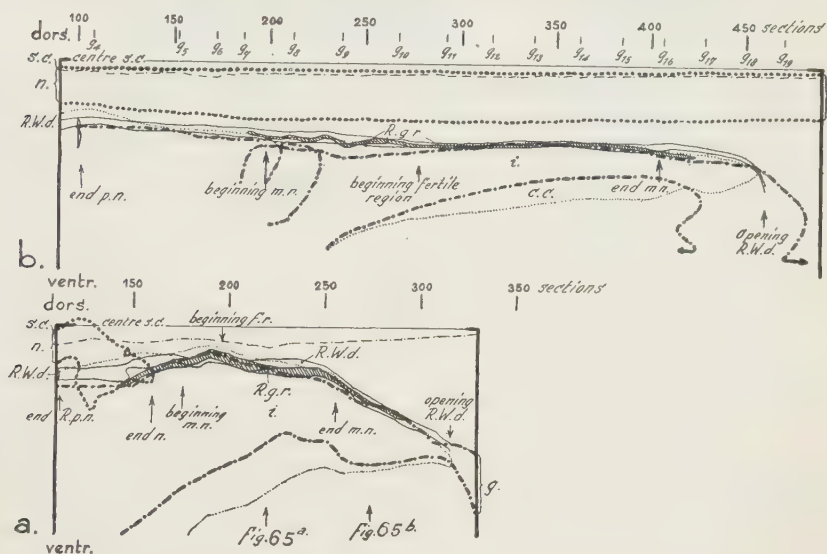


Fig. 64. a) Graphic reconstruction of trunk, *Amb. mexicanum* 304/6. Projection of right organs on sagittal plane. Vertical enlargement 25 $\times$. Horizontal: 1 section (8 μ) = $\frac{1}{4}$ mm. Outlines of organs, cf. fig. 1, p. 13. Shortened trunk. b) Id. of control *Amb. mexicanum* 215/6. Normal proportions of trunk.

many cases it bent irregularly. The small wound soon healed. In those cases where the wound did not close completely, a small intestinal fistula had been formed. The mortality was very low. Nearly all the embryos could be reared up to a stage ($\pm$ stage 43 (GLAESNER), 21 days after operation), at which the yolk material was completely consumed.

The larvae showed many abnormalities. The donors did not stretch normally, and developed into shortened larvae. Many hosts had a very hydropic character, especially the coelomic cavity being considerably enlarged. In the donors, the notochord in almost every case was entirely lacking caudally to the 5th-8th spinal ganglion, whereas the other organs had developed normally.

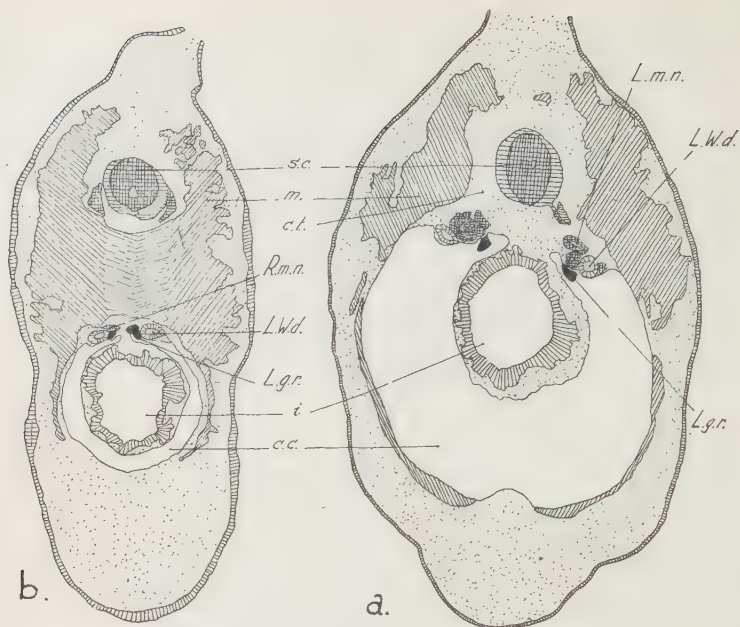


Fig. 65. Cross sections through trunk of *Amb. mexicanum* 304/6. Cf. arrows in fig. 64a. a) Middle trunk behind end of notochord, section 220. b) Posterior trunk, section 273. Enlargement $\pm 50\times$. L.m.n.: left mesonephros.

The end of the notochord was often irregular in shape. In almost every case it ended several sections before the beginning of the normally developed germ ridges. Cf. fig. 64a and b. At the place of the notochord mesenchyme cells had been formed. In the cranial part of the area of operation the intestine hung from a broad dorsal mesentery, the Wolffian duct, mesonephros and germ ridges being displaced laterally (fig. 65a). In the caudal part of the trunk the myotomes had united ventrally to the spinal cord, and formed one continuous mass of muscle cells. Here the position of the germ ridges was more normal. Cf. fig. 65b. The spinal ganglia on either side of the spinal cord had developed irregularly in the area of operation. Therefore I could not use them as landmarks for the segmentation of the trunk. The whole trunk was appreciably shortened. All organs showed a decreased length, but an increased diameter. Cf. the graphic reconstructions fig. 64a and b. The germ ridges began somewhat cranially to the mesonephros rudiments, whereas the beginning of the fertile region was situated somewhat caudally to this point (Cf.

Table 14). The germ ridges and fertile regions ended at some distance before the opening of the intestine. This corresponded entirely to the normal proportions in the controls (fig. 64b). The coelomic cavity also showed a shortened but normal form. Table 14 gives the position of the fertile region in the donors.

TABLE 14

Number of animal	Number of germ cells	Beginning of mesonephros left side	Beginning of fertile region left side	Beginning of mesonephros right side	Beginning of fertile region right side
301/2	254	section 146	section 158	section 138	section 162
301/3	230	172	184	134	156
304/5	225	208	226	206	222
304/6	206	169	192	171	196
307/8	256	194	220	197	220
307/9	188	178	190	178	186
310/1	182	125	156	116	140
310/2	298	171	184	170	184
313/4	266	144	162	170	182
313/5	297	233	232	217	230
316/7	228	184	196	180	200
316/8	238	164	156	132	158
319/20	174	198	210	173	192
319/21	315	121	136	121	132

14 × M=240

Difference M = 14

Difference M = 18

The number of germ cells has not been altered by the removal of the notochord (compare table 14, column 2, with Table 13b and c, series B, column 5).

The hosts yield more interesting results. In these animals very complicated situations appeared. I will give some examples here.

Amb. mexicanum 326/7. The graft was localized to the left of the intestine from the 12th to 19th spinal ganglion. Behind the 14th spinal ganglion, the coelomic cavity was reduced at the left side. Several supernumerary germ ridges – three fertile and two sterile – had been formed along the supernumerary notochord. Locally the intestine had been displaced somewhat to the right side, while the lateral plate mesoderm had been influenced only in the direct environment of the notochord (fig. 66 and 67a and b). The animals *Amb. mexicanum* 324/5 and probably 331/2 showed a similar structure, and contained a fairly normal number of germ cells. The distribution of the germ cells in the trunk had been disturbed by the graft. In those parts of the body where the coelomic cavity was reduced, germ ridges and germ cells were absent.

Amb. mexicanum 337/8 (fig. 68). The graft extended a great distance in the medio-ventral mesoderm from the 11th spinal ganglion to the end of the tail. The anal opening had been displaced considerably caudad by the stretching of the graft. In the first segments of the germ ridge region, viz., from the 12th to the 15th body segment, three sterile supernumerary germ ridges had been formed along the supernumerary notochord. The lateral plate

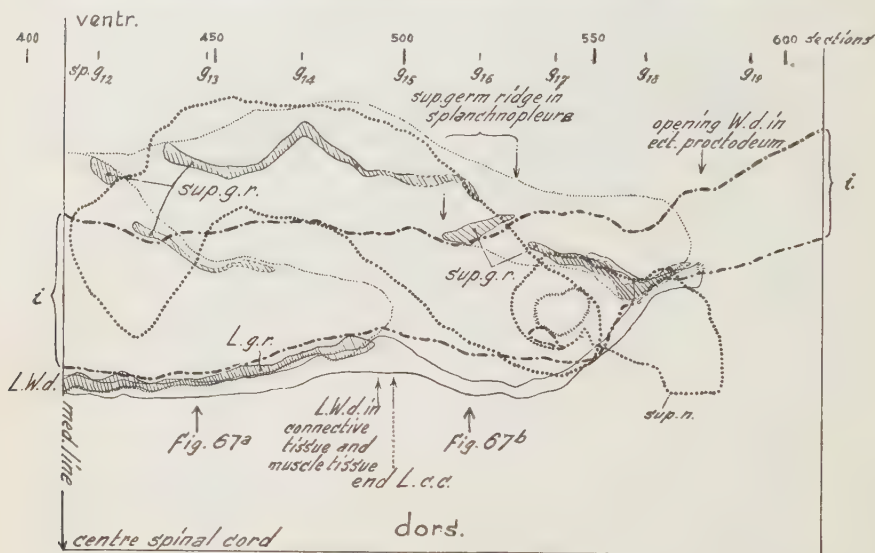


Fig. 66. Graphic reconstruction of caudal half of trunk, *Amb. mexicanum* 326/7. Projection on sagittal plane. Vertical enlargement: $\pm 50\times$. Horizontal: 1 section (8μ) = $\frac{1}{2}$ mm. Position of supernumerary (sup.) notochord and germ ridges ($5\times$); left Wolffian duct, germ ridge, intestine and coelomic cavity of host.

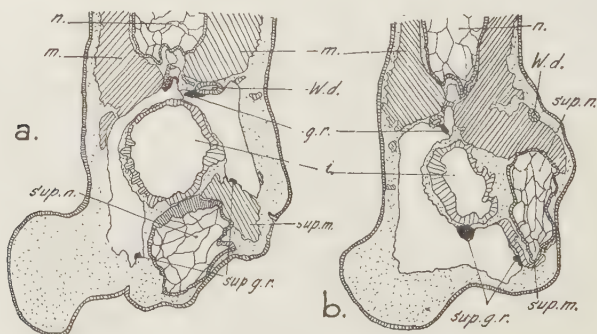


Fig. 67. Cross sections through trunk of *Amb. mexicanum* 326/7. Cf. arrows in fig. 66. a) Middle trunk, section 446. b) Posterior trunk, section 518. Position of sup. notochord, myotomes and germ ridges. Enlargement $\pm 36\times$.

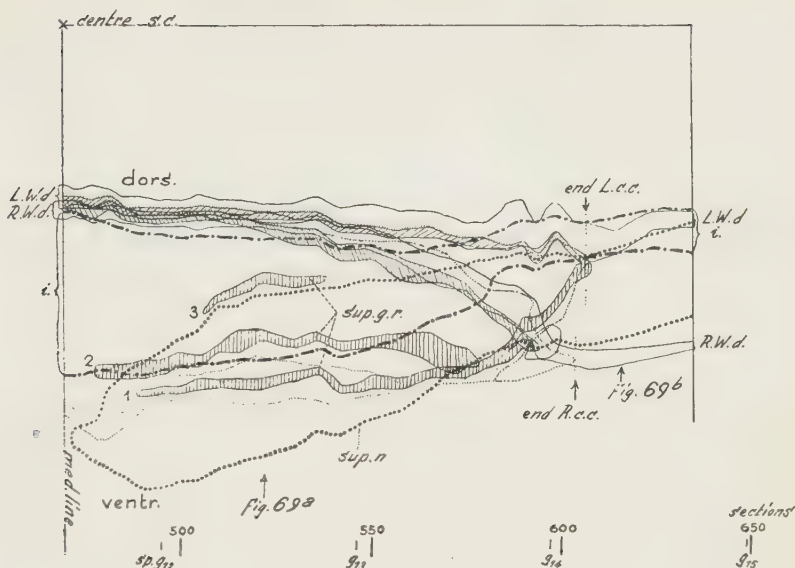


Fig. 68. Graphic reconstruction of caudal half of trunk, *Amb. mexicanum* 337/8. Projection on sagittal plane. Vertical enlargement $\pm 50\times$. Horizontal: 1 section (8μ) = $\frac{1}{2}$ mm. Position of sup. notochord and germ ridges ($3\times$); right and left Wolffian ducts, germ ridges, coelomic cavity and intestine of host.



Fig. 69. Cross sections through trunk of *Amb. mexicanum* 337/8. Cf. arrows in fig. 68. a) Middle trunk, section 522. Position of sup. notochord, myotome tissue and germ ridges ($3\times$). b) Posterior trunk, section 616. Position of sup. notochord and myotomes; Wolffian ducts and intestine of host. Enlargement $\pm 40\times$.

mesoderm had been transformed into myotome tissue in the neighbourhood of the notochord (fig. 69a). Between the 13th and the 14th spinal ganglion the diameter of the intestine decreased considerably, while the coelomic cavities also diminished rapidly in size, and ended blindly some sections behind the 14th spinal ganglion. Here the Wolffian ducts had been separated from the coelomic cavities by the enlarged myotomes. They ran laterally to the myotome mass in the subcutaneous connective tissue. The groups of muscle cells, situated along the anterior part of the supernumerary notochord in the form of irregular myotomes, fused with the myotomes of the host in the caudal part of the trunk. They formed a ring of muscle segments round the notochords and intestine; the latter had been reduced to a very small tube (fig. 69b). This animal contained only a few germ cells, situated in the anterior part of the "germinal region". *Amb. mexicanum* 333/4 and 335/6 showed similar relations.

Table no. 15 shows the situations in the hosts.

A fact to be noted is the position of the graft. As may be seen in Table 15 column 6, in some animals after the implantation the

TABLE 15

Number of animal.	Number of g.c., left side.	Number of extra regional g.c.	Number of g.c., right side.	Total number of g.c.	Position of graft with regard to spinal ganglia.	Beginning of reduction of intestine.	Number of supernumerary germ ridges.
324/5	67	22	167	256	sp. g. 12-15		1
326/7	110	42	89	241	" " 12-19		5
331/2	23	5	118	146	" " 9-12		
329/30	67	13	11	91	" " 12 to tail	sp. g. 16-17	1
322/3	6	64	17	87	" " 10-14	narrow caudal part of intestine	1
337/8	5	5	6	16	" " 11/12 to tail	sp. g. 13-14	3
333/4	1	5		6	" " 11-13	" " 12-13	1
335/6				0	" 16 to tail	strongly abnormal structure of intestine ¹⁾	1?

¹⁾ *Amb. mexicanum* 335/6 does not completely agree with the other animals of the third group. Here, the reduction of the intestine begins at the level of the 16th to 17th spinal ganglion, and the graft is localized in the most caudal part of the trunk. Under these topographical conditions, we should have expected germ cells in the cranial part of the "germinal region". This animal is, however, completely sterile. The posterior part of the mesoderm has, however, probably been displaced considerably caudad, as the ectodermal proctodaeum opens behind the level of the 23rd spinal ganglion. (Normally the anus lies at a level with the 18th to 19th spinal ganglion).

graft has hardly been displaced in cranial direction, but has retained its position near the blastopore. In the other animals, the displacements are more considerable, but still inferior to the normal displacements of the surrounding mesoderm (compare the displacements of the pronephros graft in Table 13c).

It is also of interest that groups of muscle cells and (or) nephroic tissue (pronephros tubules, side branches of the Wolffian duct, etc.) are present in the neighbourhood of the supernumerary notochord.

The most important fact, however, is that the supernumerary notochord has caused pronounced irregularities in the position of the germ layers (entoderm and mesoderm). Cf. Table 15, column 7.

Discussion.

In order to explain the structure of the various hosts, I will divide the animals into three groups. In the first group, the graft is situated ventrally or laterally to the intestine, suppressing the coelomic cavities only locally. The invagination of the lateral plate mesoderm has been little, or not at all, influenced, and the entoderm has not been displaced with regard to the mesoderm. Therefore, the determination of the p.p. germ cells took place normally. Except for irregularities in the distribution of the germ cells, and small transformations of the mesoderm in the immediate neighbourhood of the notochord, these larvae showed normal proportions. *E.g. Amb. mexicanum* 326/7. Cf. Table 15, group I. In the second group conditions intermediate between the first and the third group are found. In the third group, however, the graft has exercised a great influence upon the adjacent tissues, and has considerably displaced the entoderm with respect to the mesoderm, so that very abnormal situations occur. *E.g. Amb. mexicanum* 337/8. Cf. Table 15, group III.

How are we to explain the greatly reduced number of germ cells and the abnormal structure of the animals of the third group?

Two different phenomena which have altered the normal development may be distinguished.

1) As shown by my own observations on the early developmental stages of experimental animals of this and similar transplantation series (transplantations of the dorsal blastoporal lip), the entoderm mass has been displaced by the graft with regard to the mesoderm. At the neurula stage, the presence of the notochord graft has impeded the invagination of the ventral and

latero-ventral mesoderm which, therefore, has accumulated in the blastoporal lips. In this way the caudal entoderm and the presumptive lateral plate mesoderm have been separated from each other. A long pear-shaped embryo has been formed. Then the accumulated mesoderm in the ventral blastoporal lip has been pushed away caudad, whereas the caudal entoderm has been displaced in the opposite direction by the stretching of the graft. In this way the caudal entoderm and mesoderm have been separated definitively.

2) The presumptive lateral plate mesoderm in the neighbourhood of the blastopore has for the greater part been transformed into myotome and mesenchyme tissue (cf. preceding pages). In the caudal part of the embryo, the entoderm has been reduced to a very narrow strip of cells. Here, the axial organs of the host may also have influenced the differentiation of the presumptive lateral plate mesoderm.

The first of these malformations has prevented the induction of the p.p. germ cells, as a direct contact between the dorso-caudal entoderm and the lateral plate mesoderm is necessary to the determination of the germ cells. The second has prevented their differentiation, as the germ cells cannot differentiate under altered inductive influences. For these reasons the great decrease in the number of germ cells is comprehensible. In these experimental animals both influences have acted. They can hardly be distinguished from each other in the individual cases.

Consequently the few germ cells present were localized in the cranial part of the "germinal region" only. This lateral plate mesoderm had already been invaginated, and had not afterwards been transformed by the graft.

In the second group, these phenomena are much less pronounced, so that these animals show more normal topographical relations and higher numbers of germ cells. Cf. Table 15, group II.

The fusion of the right and left myotomes ventrally to the spinal cord after the removal of the notochord entirely agrees with the investigations of KITCHIN (1938).

The graft has been carefully freed of adhering mesodermal cells. Moreover, any attractive action of the notochord upon muscle fibres has never been observed. Therefore, the myotomes and nephroic tissue along the supernumerary notochord must originate from transformed cells of the lateral plate mesoderm (YAMADA (1937-'39)). Hence, the notochord must have actively influenced the differentiation of the surrounding mesoderm. Cf. BAUTZMANN (1928 and '29).

C. GENERAL DISCUSSION

(Supplement to p. 149-153)

From the extirpation experiments we may conclude that the determination and differentiation of the germ cells (within the "germinal region") may take place normally in the absence of the notochord, or Wolffian duct. The inductive action of the entoderm is evidently sufficient for the determination of the presumptive germ cells. Moreover, the regional induction along the longitudinal axis of the embryo has not been influenced by the absence of these organs.

I had found in my heteroplastic transplantation experiments that the germ cells cannot differentiate in abnormal positions (outside the lateral plate tissue). This phenomenon was explained by assuming a depressing influence upon the differentiation of the presumptive germ cells by other inductive actions. The last series of experiments supports the opinion that the notochord may actually prevent the differentiation of the germ cells. It is not yet clear, however, whether these presumptive germ cells have been transformed into somatic cells, or have degenerated under such conditions. These series also prove that the determination of the p.p. germ cells in the presumptive lateral plate mesoderm can also be prevented, either by the removal of the whole entoderm, or by the mutual displacement of entoderm and mesoderm.

HUMPHREY (1925) stated that "the p.p. germ cells are specific elements, which do not belong to one of the germ layers, and which may not be predetermined, but may differentiate from entoderm or mesoderm under the influence of various environmental conditions." With this exception no authors have presupposed the possibility of an inductive action in the development of the germ cells. My investigation casts an entirely new light upon the development of the germ cells. It shows that a determinative action is indispensable to their final differentiation, notwithstanding the possibly predetermined state of the p.p. germ cells. This inductive action is exerted by the dorso-caudal entoderm. As far as I have been able to show, it cannot be replaced by influences from other tissues (pronephros, Wolffian duct and notochord). Hence, this induction, too, is most probably specific.

PART II. CHAPTER 3
THE MIGRATION OF THE GERM CELLS IN THE
URODELES.

GENERAL DISCUSSION.

In the course of development we may observe a migration of the germ cells. At an early tail-bud stage they are accumulated in the neighbourhood of the Wolffian duct in the most dorsal part of the lateral plate. Then, in the early larva they form a median germ cell ridge, situated ventrally to the notochord, and finally, in the older larva, they fill up the two definitive germ ridges.

In my opinion there are no indications to consider these accumulations of germ cells, in the neighbourhood of the Wolffian duct and in the median plane, as anything else than temporary intermediate stations in the migration process.

In order to analyse the migration process, I will divide the migration of the germ cells from their place of origin to their definitive position into three phases. The p.p. germ cells migrate in the first phase from their place of origin to the neighbourhood of the Wolffian duct; in the second, from here to the median plane; and in the third, from the dorsal germ cell ridge to the definitive germ ridges.

The question which engages us here is whether this migration is active or passive. As we have seen in the survey of the literature, there are three divergent opinions; 1) active migration; 2) partially active, partially passive migration; 3) passive migration (caused by growth processes in the surrounding tissues). Which of these hypotheses is applicable to the migration process in the Urodeles?

First phase. We now know that the germ cells originate from the presumptive lateral plate mesoderm, where they are probably scattered among the cells of the whole lateral plate. (From all the parts of the lateral plate within the germinal region, germ cells derive in fairly equal numbers). At the end of the first phase they are accumulated in the dorsal part of the lateral plate in the neighbourhood of the Wolffian duct.

Two questions arise, *viz.*: In what manner do the germ cells migrate to the dorsal part of the lateral plate? and: Why do they accumulate in the neighbourhood of the Wolffian duct?

The presumptive lateral plate mesoderm lies in the ventral and

latero-ventral blastoporal lips at the early yolk plug stage. As the vital staining experiments have shown, this material moves externally along the meridians to the blastopore. After the invagination round the blastoporal margin, however, it diverges to the right and left, and shifts to the lateral sides by the dorsal convergence movements. In this way it covers the lateral sides of the central entoderm mass at the late neurula up to an early tail-bud stage. Finally, during the stretching of the embryo, the lateral plate mesoderm extends in a ventral direction – simultaneously, the broad area of the blood island acquires an elongated shape. At this stage the lateral plate mesoderm covers the ventral to lateral side of the entoderm of the early larva (except the medio-ventral strip of mesoderm, the presumptive blood cells). We see, therefore, that at first the p.p. germ cells may be displaced passively by the normal gastrulation and neurulation movements from their place of origin to the lateral sides of the mesoderm, ventrally to the nephrogenous cord and Wolffian duct.

During the ventral convergence movements of the lateral plate mesoderm, an active migration of the germ cells must be assumed. It may be that the germ cells are first displaced passively with the mesoderm to the ventral side, and then migrate actively to the Wolffian duct. It is, however, much more likely that an active migration of these cells to the dorsal side takes place during earlier stages at which the lateral plate mesoderm still forms a narrow band of cells in the lateral mesoderm. At these stages a migration of the germ cells for a short distance would suffice for them to reach the most dorsal portion of the lateral plate mesoderm, which is not displaced secondarily to the ventral side.

This active migration must be directed by an attractive action. For such an attraction only the Wolffian duct, the nephrogenous cord and the myotomes come into consideration. As the accumulation of the germ cells is strictly localized, an attractive action from the large myotomes is not probable. Therefore, there remain only the Wolffian duct and the nephrogenous cord to be considered. It is not yet possible, however, to decide, which of these two exerts this attraction. It was observed that after the removal of the whole entoderm at the early neurula stage the germ cells lie in the neighbourhood of the Wolffian duct and nephrogenous cord – definitive germ ridges have not been formed in these embryos, cf. the third part of this publication.

This experiment also indicates that these organs attract the germ cells.

In the second phase, the germ cells migrate from the neighbourhood of the Wolffian duct to the median plane, where they form the median germ cell ridge. ¹⁾ During this period of development, the dorsal part of the lateral plate actively grows out to the median plane. Hence it is obvious that the displacement of the germ cells from the neighbourhood of the Wolffian ducts to the median plane is mainly, or wholly, a passive one.

Moreover, what organ could attract the germ cells here? In my experiments I never observed an attractive action of the notochord. A mutual attraction between the germ cells of right and left sides is very improbable. In that case a mingling of the germ cells of the right and left sides in the median germ cell ridge might certainly be expected.

In the third phase, an active migration is again very probable. In the transplantation experiments of pronephros and notochord, the supernumerary germ ridges having been formed along the supernumerary Wolffian duct and notochord, often contain germ cells – although in small number –, whereas in their neighbourhood germ cells are entirely absent. We may, therefore, conclude that the definitive germ ridges exert an attractive influence upon the germ cells. Moreover, it is difficult to recognize in what manner the germ cells could be transported passively. Extensive displacements of cell material do not occur in this region during this stage of development.

We may transfer these considerations concerning the Urodeles to the Anures. Then, the migration of the germ cells within the entoderm may be assumed to be an active one. The separation of the dorsal germ cell ridge from the entoderm may occur passively by the growth of the dorsal parts of the lateral plates to the median plane. Finally, the migration of the germ cells to the definitive germ ridges will again represent an active displacement. This opinion agrees with the views of PERLE (1927) and CHENG (1932).

¹⁾ As HUMPHREY (1927) has already proved, the right and left germ cells do not mingle in the germ cell ridge, so that the latter essentially consists of two separate halves, a right and a left one. In my heteroplastic transplantation experiments of the presumptive lateral plate mesoderm in some cases but one germ ridge contains foreign germ cells (often in a large number). I can therefore fully confirm this statement.

SUMMARY OF PART II A, B AND C.

1) The morphological literature concerning the origin of the germ cells in the Vertebrates can be grouped as follows:

a) The germ cells have a purely entodermal origin in early development.

b) They originate from the mesoderm in early development.

c) They derive from the germinal epithelium.

d) They derive from embryonic entoderm and germinal epithelium.

e) They originate from embryonic mesoderm and germinal epithelium.

Many investigations point to an early entodermal origin of the germ cells in the Anures, although the theory of a second generation of germ cells from the germinal epithelium is defended by several authors. Respecting the Urodeles, the opinions of the various authors are altogether conflicting.

2) The experimental literature is in favour of an early entodermal origin of the germ cells in the Anures, and of a possible early mesodermal origin in the Urodeles. As to a secondary origin of germ cells from the germinal epithelium, the experiments are contradictory.

3) My own experiments were carried out with Urodeles (*Triturus* species and *Ambystoma mexicanum*).

4) Vital staining experiments of the entoderm yielded results completely in agreement with those of VOGT, SCHECHTMANN, and others. The material, situated at the vegetative pole of the uncleaved egg, ascends to the blastocoel. It furnishes the ventro-caudal entoderm, which will form the nutritive yolk material. The superficial cells of the ventral half of the vegetative field of the gastrula develop into the dorso-caudal entoderm, which will form the entodermal epithelium of the intestine.

5) Removal of the plasma at the vegetative pole of the uncleaved egg has no influence upon the formation and differentiation of the germ cells. The same holds good when this material is removed at the early gastrula stage by defects in the bottom of the blastocoel.

6) Removal of the superficial cell layers of the ventral half of the vegetative field at the early gastrula stage causes a great reduction in the number of germ cells, accompanied by an impaired differentiation of these cells. The fertile region decreases in length with a decreasing number of germ cells. The distribution

of the germ cells along this fertile region, however, remains regular.

7) Vital staining experiments of the posterior part of the mesoderm prove that the presumptive lateral plate mesoderm occupies the ventral and latero-ventral blastoporal lips at the early yolk plug stage. The nephrogenous cord mesoderm, situated for the greater part in the ventro-lateral blastoporal lips, forms a horse-shoe-shaped band of cells round the presumptive lateral plate mesoderm.

The presumptive lateral plate mesoderm diverges considerably to the lateral sides after passing the ventral blastoporal lip, and shifts dorsad by the movements of dorsal convergence. It occupies a narrow strip of cells at the lateral sides of the entoderm mass at the neurula stage. During the stretching of the embryo, it extends in a cranial and ventral direction (ventral convergence), and finally covers nearly the whole entoderm.

8) After removal of the presumptive lateral plate and nephrogenous cord mesoderm at the early neurula stage these parts are partially regulated from more dorsal mesoderm. Notwithstanding this regulation, several embryos show a complete absence of germ cells. The germ cells, left behind after incomplete sterilization, show a good differentiation, and are localized in the most cranial part of the "germinal region". Removal of the presumptive myotome mesoderm causes no alteration in the number and differentiation of the germ cells.

9) These observations prove that both the dorso-caudal entoderm and the posterior mesoderm (lateral plate and nephrogenous cord) have a rôle in the formation of the germ cells. The distribution and differentiation of the germ cells in these experiments are factors which favour the opinion of an origin of the germ cells in the mesoderm, and the presence of an inductive centre in the dorso-caudal entoderm.

10) After the removal of the whole entoderm at the early neurula stage, the larvae show a reduced number of germ cells localized in the cranial segments of the germinal region. Only that part of the mesoderm contains germ cells which was already invaginated at the time of operation and has, therefore, been in close contact with the dorso-caudal entoderm. These facts are an additional motive for assuming an inductive action between entoderm and mesoderm.

11) In the Urodeles the size, stainability, etc., cannot be used

as species characters of the germ cells in heteroplastic transplantation experiments. Only the pigmentation of the germ cells forms a reliable difference between the germ cells of *Tr. cristatus* and the other Urodele species used.

12) After heteroplastic transplantation of the whole entoderm, the animals show only germ cells of that species which furnished the ecto- and mesodermal layers. The germ cells, reduced in number, are situated chiefly in the cranial half of the "germinal region". Their number and position cannot be explained merely by the inductive action of the host entoderm before the operation, but constrain us to assume a supplementary induction by the grafted entoderm.

13) Heteroplastic transplantations of the presumptive lateral plate and nephrogenous cord mesoderm prove that myotomes, Wolffian ducts, nephrogenous cords and entoderm do not form sources of germ cells. They prove that the germ cells originate from the presumptive lateral plate mesoderm only. This series lends again (cf. no. 8) support to the opinion that lateral plate tissue within the germinal region, formed by regulation from other mesoderm, cannot form p.p. germ cells. This fact is an argument in favour of a predetermination of the presumptive germ cells.

14) This predetermination may be brought about by the segregation of a special sort of protoplasm during the "chemo-differentiation" of the egg. This implies probably that only the presumptive germ cells have "reactive power" to the inductive stimuli exerted by the entoderm; in other words, the presumptive germ cells are already specific elements. Cf. BOUNOURE 1939.

15) Vital staining experiments of entoderm and mesoderm prove that the dorso-caudal entoderm and the presumptive lateral plate mesoderm come into direct contact with each other at the end of the gastrulation and during the first half of the neurulation period, so that an induction can actually take place.

16) Germ cells differentiate in the presumptive lateral plate mesoderm, invaginated a few hours before the removal of the entoderm at the early neurula stage. This fact proves that the determination of the germ cells occurs very rapidly.

17) Transplantation experiments of the pronephros rudiment at the early neurula stage prove that the Wolffian duct grows out from the caudal part of the pronephros rudiment to the cloaca,

and that every cell of the Wolffian duct rudiment has the power of active migration.

18) Probably, neither the pronephros nor the Wolffian duct influences the formation and differentiation of the germ cells.

19) Transplantation experiments of the notochord render it probable that the notochord may even prevent the differentiation of the germ cells in its environment.

20) The fact that several organs from which inductive influences emanate and act upon other parts (notochord, Wolffian duct), cannot replace the action of the dorso-caudal entoderm in the determination process of the germ cells, points to a specific character of this action.

21) A disturbance of the invagination of the mesoderm by an active influence of a supernumerary notochord, coupled with a mutual displacement of the caudal entoderm and mesoderm by the stretching of the graft, had prevented the contact between caudal entoderm and mesoderm in several animals. In these animals the presumptive germ cells could not be determined. Moreover, the transformation by the notochord of the adjacent lateral plate mesoderm has prevented the germ cell differentiation.

22) Three phases in the germ cell migration in the Urodeles can be distinguished. In the first phase, they migrate from their place of origin to the neighbourhood of the Wolffian duct, in the second from this point to the median plane, and in the third, from the median germ cell ridge to the definitive germ ridges.

23) The displacements of the germ cells in the first phase occur, probably, by a combined active and passive migration. The first displacements are mainly passive (gastrulation movements), whereas the end of this migration path is covered by an active migration of the germ cells. In the second phase, a passive transport of the germ cells by the growing out of the dorsal parts of the lateral plates suffices to explain the displacements of the germ cells. Finally, in the third phase, we may conclude from the absence of extensive material displacements and the presence of an attractive action from the definitive germ ridges, that the migration is active again.

PART III. THE DETERMINATION OF THE DEFINITIVE GERM RIDGES IN THE URODELES.

A) INTRODUCTION, AND DESCRIPTION OF THE NORMAL DEVELOPMENT OF THE GERM RIDGES.

The experimental investigations on the origin of the germ cells brought to light many interesting facts concerning the determination of the germ ridges. I will record them in this part of the paper.

The definitive germ ridges develop from the dorsal part of the coelomic wall. Between the Wolffian duct and the dorsal mesentery, on both sides of the median plane, an accumulation of peritoneal cells appears, which forms a longitudinal fold in the peritoneum. This fold grows out and forms the germ ridge rudiment. After the appearance of the mesonephros, rete cords, consisting of nephrogenous cord cells, grow into the germ ridge, and fill up the so-called "primary genital cavity". (This is, however, no true cavity, but a space filled by very wide-meshed mesenchyme.) In this way the definitive germ ridge is formed.

All the experimental animals of the various series have been fixed approx. at stage 43 (GLAESNER). At this stage the germ ridges form but narrow folds, U-shaped or pear-shaped in cross section. Cf. Pl. VII, p. 128 and fig. 65, p. 162. Therefore, only the initial stages of development of the definitive germ ridges have been considered.

In the literature the authors agree in admitting a peritoneal origin of the early germ ridge rudiment.

B) EXPERIMENTAL PART.

a) Nature of the determination process.

Experiments and results.

My experiments show clearly that a germ ridge can develop only from the peritoneum of the coelomic wall. In those parts of the body where abnormalities have prevented the formation of a coelomic cavity, the germ ridges are lacking. In the notochord transplantations (cf. p. 163-166), the hosts often showed abnormalities in the formation of the coelomic cavity. In *Amb. mexicanum* 337/8, the right and left coelomic cavities ended some sections behind the level of the 14th spinal ganglion (fig. 68, p. 165). On the

right side, the germ ridge terminated about 10 sections before the end of the coelomic cavity, while on the left the germ ridges and the coelomic cavity ended even in the same section. In all my experimental animals, I never found any indication of the formation of a germ ridge outside the coelomic cavity. We may therefore conclude that the germ ridges are dependent upon the presence of the peritoneal epithelium, and cannot be formed from any other tissue.

The experiments in which the whole entoderm was removed at the early neurula stage, cf. p. 119, reveal an interesting fact concerning the determination of the germ ridges. In all animals of this series, the germ ridges were lacking. The objection that the embryos were still too young at the time of fixation, can be rejected; they have been reared even as long as 32 days after operation, at which age the germ ridges in normal animals have always been formed. Fig. 70 shows that in spite of the normal position of the Wolffian ducts beneath the peritoneum of the coelomic cavity, no germ ridges have been formed. In this case the mutual position of coelomic cavity and notochord

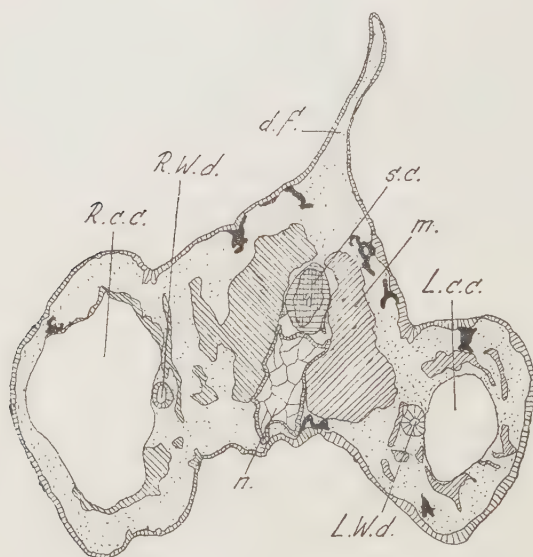


Fig. 70. Cross section through trunk of *Tr. taeniatum* 369, section 12. Complete absence of germ ridges after removal of whole entoderm at early neurula stage. Enlargement $66\frac{2}{3}\times$.

differs from that in normal animals. In the entoderm-free animals of series 1' – the animals were flattened medio-laterally (cf. p. 18-19) – the germ ridges were also lacking. Here the position of the coelomic cavity with respect to Wolffian ducts, mesonephros and notochord was quite normal. Therefore we are forced to conclude that the germ ridges have failed to appear owing to the absence of the entoderm. We can explain this result only by assuming that the entoderm activates the peritoneum in a general way, evoking in the whole peritoneum a "reactive power" (RAVEN (1938)). This reactive power enables the peritoneum to react to local inductive stimuli by the formation of germ ridges. This general

activation is, therefore, indispensable to the formation of the germ ridges. It is, however, insufficient for their differentiation.

As possible sources of the local inductive stimuli mentioned above, bringing about the local determination of the germ ridges, several adjacent organs may be considered; viz., the Wolffian duct, the notochord, the mesonephros, the germ cells, the myotomes and the entoderm. All these organs are situated in the neighbourhood of the presumptive germ ridges.

We will first consider the transplantation of the pronephros and Wolffian duct rudiment, the method of operation of which having been described above, p. 153.

Several donors in which, besides the pronephros rudiment, also the Wolffian duct was absent, showed a normal germ ridge on the operated side (fig. 71). It contained a number of germ cells almost equal to that of the non-operated side. Evidently the Wolffian duct is not indispensable to the formation of the germ ridge.

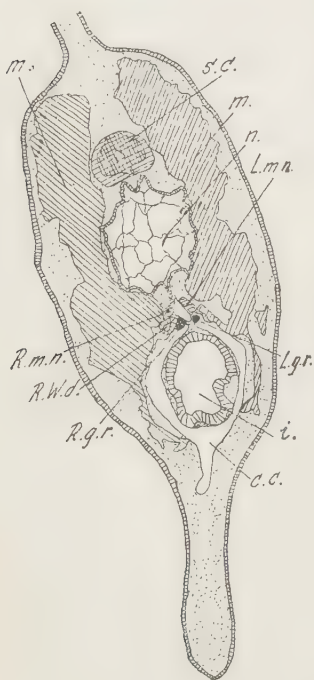


Fig. 71. Cross section through caudal trunk of *Amb. mexicanum* 168/9 (XX), section 398. Development of germ ridge and mesonephros with complete absence of Wolffian duct on operated side (L.). Enlargement $66\frac{2}{3}\times$.

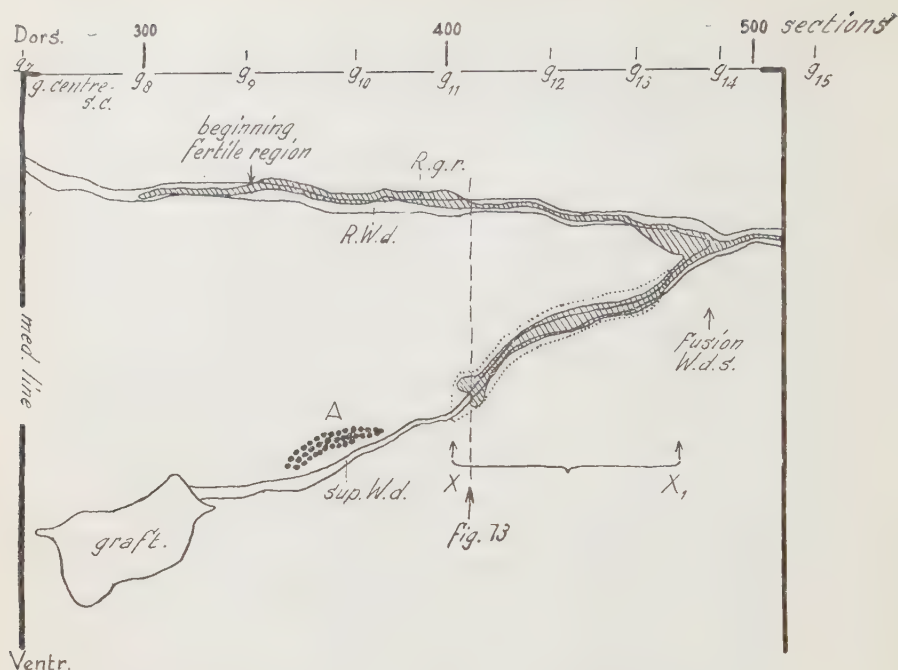


Fig. 72. Graphic reconstruction of trunk of *Amb. mexicanum* 286/7. Projection on sagittal plane. Vertical: Enlargement $\pm 40\times$. Horizontal: 1 section (8μ) = 0,4 mm. Position of sup. pronephros, Wolffian duct and germ ridge; R. Wolffian duct and germ ridge of host. X-X': Course of Wolffian duct through coelomic cavity. A: Isolated group of germ cells in ventral somatopleure.

As Table 13c, p. 157, shows, in 4 hosts a supernumerary Wolffian duct had grown out from the grafted pronephros rudiment. In three cases along the supernumerary Wolffian duct a supernumerary germ ridge had developed. Figs. 72 and 73 (*Amb. mexicanum* 286/7) demonstrate clearly the development of such a supernumerary germ ridge, containing a small number of germ cells. This germ ridge, which fuses with that of the host posteriorly, has been formed along that part of the Wolffian duct which passes through the coelomic cavity. Along the Wolffian duct no mesonephros tubules or other organs have been formed. In another animal, the supernumerary germ ridge was situated in the splanchnopleure; the Wolffian duct ran through the splanchnopleure after passing the coelomic cavity.

We may conclude from these observations that the Wolffian

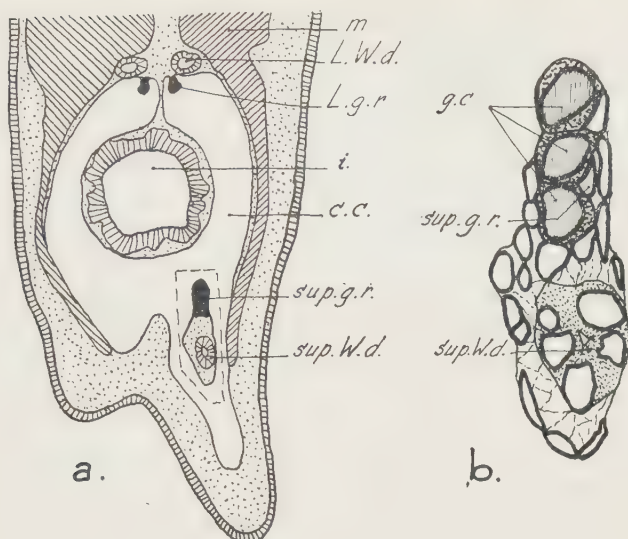


Fig. 73. a) Cross section through caudal trunk region of *Amb. mexicanum* 286/7. Cf. arrow in fig. 72. Enlargement $60\times$. Position of sup. Wolffian duct and germ ridge. b) Detail, enlargement $240\times$.

duct can determine the local differentiation of a germ ridge in a heterotopic position in the peritoneal epithelium of both somatopleure and splanchnopleure. The fact that the supernumerary germ ridges never begin at a more cranial level than those of the host, indicates that there is also a "germ ridge region" in the trunk. This region is somewhat larger than the germinal region, and is probably also determined by a regional induction along the longitudinal axis of the trunk.

The transplantation of the notochord yields similar results. The operation method has been described on p. 160. As fig. 65, p. 162, shows, in the donors the differentiation of the germ ridges takes place independently of the absence of the notochord in the caudal half of the trunk. In the considerably shortened trunk, short but broad germ ridges containing many germ cells, have been formed, and normally differentiated in the neighbourhood of the Wolffian duct and mesonephros rudiments. Hence, the notochord is neither indispensable to the formation of the germ ridges.

The hosts showed several supernumerary germ ridges in the peritoneal epithelium in the neighbourhood of the grafted noto-

chord. Fig. 68, p. 165, clearly demonstrates their positions along the graft. These germ ridges contain only a few germ cells. One of the germ ridges, *viz.*, no. 1 fuses posteriorly with the right and left germ ridges of the host. Along part of this germ ridge myotomes have developed in the triangular space between notochord, skin and coelomic cavity. The second is an isolated longitudinal ridge in the right somatopleure at the ventral end of the muscle-plate, while the third is situated in the splanchnopleure. Fig. 69a, p. 165, shows these topographical positions. Since the animal contained hardly any germ cells, these supernumerary germ ridges were sterile. This figure also shows that the germ ridges have been formed in the pleures at some distance from the notochord.

We must conclude, therefore, that the notochord can also determine the local differentiation of germ ridges in the activated peritoneum. The supernumerary germ ridges, induced by the supernumerary notochord, never begin at a level anterior to the beginning of the normal germ ridges of the host. These first show no topographical relations to the grafted notochord, but exclusively to the topography in the trunk of the host. We may assume that the notochord itself does not determine the regional germ ridge formation.

The significance of the mesonephros has not been investigated experimentally.

In the experimental animals in which the presumptive lateral plate and nephrogenous cord mesoderm had been removed at the early neurula stage (cf. p. 105), normal germ ridges were formed in the absence of p.p. germ cells (cf. fig. 38, p. 106 and fig. 39, p. 106). Hence, the p.p. germ cells are neither necessary to the formation of the germ ridges. The direct influence of the germ cells upon the activated peritoneum has, however, not been investigated experimentally.

Discussion.

The transplantation experiments show that both the Wolffian duct and the notochord may induce the local differentiation of germ ridges in the peritoneal epithelium. Evidently the determination of the germ ridges is safeguarded by double insurance. The negative results of the removal of either Wolffian duct or notochord can now be easily explained. In both cases one of the inductors remained, and determined the formation of the germ ridges.

I often observed in my experimental animals that the germ ridge was absent in those parts of the trunk where the Wolffian duct had been separated from the coelomic epithelium -- *e.g.* by the presence of a graft -- and was situated at some distance from it. This fact also indicates that there is a close relation between Wolffian duct and germ ridge.

The observation of muscle or (and) nephric tissue in the neighbourhood of the supernumerary notochord may give rise to the objection that a secondary induction from these tissues may perhaps have taken place in these cases of supernumerary germ ridge formation. However, in many animals there were no traces of nephric tissue or myotomes along the supernumerary germ ridges, so that a direct induction by the notochord must be assumed here.

The supernumerary germ ridges situated along the supernumerary Wolffian duct or notochord contained but a small number of germ cells. This is probably due to the delayed differentiation of these germ ridges -- as may be concluded from their much smaller size. The attractive action of the further differentiated host germ ridges upon the germ cells exceeds that of these supernumerary germ ridges.

The removal of the mesonephros rudiment presents too great difficulty, and cannot be performed without considerable injury to the adjacent organs.

The different views held concerning the relations between Wolffian duct, mesonephros and germ ridge in several Urodelian and Anurian species may probably be explained as follows: We may presuppose that there are various strengths of the inductive action from the Wolffian duct on one hand, combined with various intensities of the self-differentiation tendencies of the mesonephros rudiment itself on the other in the separate species. MIURA's observations (1939) in *Rana*, and GRÜN WALD's investigations (1937) in the chicken, show that in some species the development of the mesonephros is probably entirely dependent upon the presence of the Wolffian duct. In *Rana* (MIURA (1939)) the mesonephros failed to appear after the extirpation of the Wolffian duct rudiment, but the early germ ridge rudiment showed a normal development. According to MIURA, the development of the gonads is independent of the presence of the mesonephros rudiments up to the larval stage, at which the rete cords enter the gonads. Then a dependent differentiation begins. In GRÜN WALD's experiments, the proliferation of the coelomic epithelium

of the gonad took place normally in the absence of the mesonephros. The lack of these proliferations in DANTSCHAKOFF's experiments (1933) is ascribed by GRÜNWALD to injuries caused by the Röntgen radiation. MIURA's observations are partly contradicted by SHIMASAKI (1930), while GRÜNWALD's results do not entirely agree with DANTSCHAKOFF's experiments. Nevertheless, it is at least probable that in some species the germ ridge may appear in the absence of the mesonephros. On the other hand, we may suppose that the mesonephros rudiments are not wholly devoid of influence upon the formation of the germ ridges. Whether the mesonephros rudiments can induce supernumerary germ ridges in a heterotopic position, however, remains to be studied.

The same consideration holds for the influence of the p.p. germ cells. Although my experiments clearly show that the p.p. germ cells are not necessary to the formation of the germ ridges, it is not yet known whether the p.p. germ cells can induce a supernumerary germ ridge. The sporadic observations in the literature of extra-regional gonads, developed round isolated groups of germ cells in heterotopic positions, may point to an active influence of the p.p. germ cells.

Finally there remains the possibility of a local inductive action of the myotomes or the entoderm. In my opinion, such an action is very improbable, as these organs over a large area border the coelomic cavity, so that a local action is out of the question.

Surveying these transplantation experiments, it becomes clear that the influence of the entoderm is merely a general one. In the entoderm-free animals, the local inductive influences of notochord, Wolffian duct, etc., were normally present, but nevertheless germ ridges did not develop.

This fact throws an interesting light upon this determination process. The entoderm awakens "reactive powers" to germ ridge formation in the whole peritoneum, but any "differentiation tendency" has not yet appeared; hence, in the absence of further inductive stimuli no germ ridges are formed.

We can therefore divide the determination of the germ ridges into two phases, *viz.*, 1) the general activation (evocation of reactive powers) of the whole peritoneal epithelium by the entoderm, and 2) the local induction of the germ ridge in the activated peritoneum by

the Wolffian duct, notochord and, it may be, by mesonephros and p.p. germ cells. Owing to these influences differentiation tendencies appear.

The succession of "reactive powers" and "differentiation tendencies" in this determination process entirely agrees with RAVEN's views (1938). According to RAVEN, the reactive powers increase during the determination process, and gradually change into differentiation tendencies. The fact that germ ridges are entirely lacking in the absence of entoderm, notwithstanding the presence of an inductive action from notochord and Wolffian duct, has interesting consequences, however. We can explain this fact in two ways. 1) The inductive influence of notochord and Wolffian duct is of the same nature as that of the entoderm. It is, however, too weak to awaken a strong reactive power, and to convey it into a differentiation tendency in inactivated peritoneum. The previous activation of strong reactive powers by the entoderm is, therefore, indispensable. The actions of entoderm and local inductors may be counted together. It might be imagined in this case that the previous action of the entoderm could be replaced by an increase in the strength of the local inductors. 2) The second phase of the determination process differs qualitatively from the first; the nature of both inductive stimuli is different. The peritoneum, therefore, cannot react upon the induction of notochord and Wolffian duct unless it has previously been activated by the entoderm. The two induction processes cannot relieve each other; their order cannot be reversed; they are qualitatively different links in the causal chain. The reactive powers, provoked by the entoderm, cannot simply pass into differentiation tendencies. Perhaps, an essential difference exists between the real nature of the reactive powers and of the differentiation tendencies themselves.

The formation of many supernumerary germ ridges along a grafted notochord, points to the presence of strong reactive powers in the activated peritoneum on one hand, but also to a strong inductive influence of the notochord on the other. We cannot yet decide between these two possibilities; a further investigation of this question may furnish important contributions towards a better understanding of this determination process.

b) The regional determination of the germ ridges.

Series 16. Transplantation of the entoderm-free ecto-mesodermal envelope of embryo I into the ventral side of embryo II at the early neurula stage.

Experiment and results.

Operation method. Cf. fig. 74. From the smaller embryo of *Tr. taeniatus* the entoderm was removed in the usual way

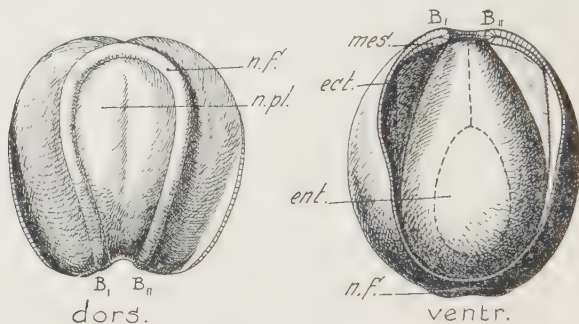


Fig. 74. Sketch of antero-posterior transplantation of ecto-mesodermal envelope of *Tr. taeniatus* neurula in ventral side of *Tr. alpestris* neurula. a) Ecto-mesodermal envelope, dorsal view. b) Preparation of wound field in *Tr. alpestris* neurula; ventral view. Medio-ventral cut through ectoderm and mesoderm; removal of ectoderm of mesoderm-free area; separation of ectoderm and mesoderm from central entoderm mass. Enlargement 15-20 $\times$. B'-B'': opened blastopore.

(cf. p. 10-11), and the ecto-mesodermal envelope spread out. In the larger embryo of *Tr. alpestris* a medio-ventral cut was made through the ectoderm and underlying mesoderm from the blastopore to the cranial neural fold. Then the ectoderm and mesoderm were separated for a considerable portion from the central entoderm mass. The ectoderm of the mesoderm-free area was finally removed.

The ecto-mesodermal envelope of *Tr. taeniatus* was grafted into the large wound field in a reversed position, with its cranial side upon the caudal, and with its spread blastoporal lips upon the cephalic, entoderm of the host. Pressed gently against each other by a glass bridge, the embryos grew together, and an reverse duplicitas embryo was formed.

This series consisted of three animals only. They developed rather well. The hosts showed a normal external form; the grafts

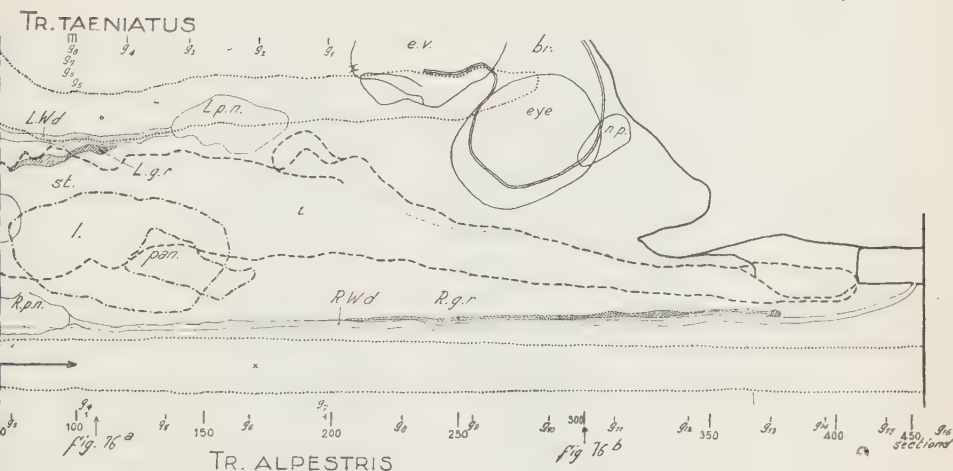


Fig. 75. Graphic reconstruction of trunk of *Tr. alpestris/taeniatus* 494. Inverse duplicitas. Projection on sagittal plane. Vertical enlargement $33\frac{1}{3}\times$. Horizontal: 1 section (8μ) = $\frac{1}{3}$ mm. Position of L. Wolffian duct and germ ridge of *Tr. taeniatus* embryo with regard to intestine, R. Wolffian duct and germ ridge of *Tr. alpestris* embryo. St.: stomach, n.p.: nasal pit, br.: brain.

were microcephalic, their tails often bent considerably dorsad. They were fixed 27 days after operation.

The caudal part of the trunk of the grafted *taeniatus* embryo, which bordered upon the cephalic region of the *alpestris* host, had altered in shape and differentiation. The cephalic organs of the host had strongly influenced the lateral plate mesoderm of the graft. A coelomic cavity had seldom been formed. The lateral plate mesoderm had for the greater part been assimilated in the cephalic mesoderm of the host. Only one of the three animals of this series, *Tr. alpestris/taeniatus* 494, showed a fairly normal coelomic cavity in the middle trunk region of the graft. This case is very interesting.

Fig. 75 shows the position of the graft with regard to the host. The *taeniatus* head is situated on a level with the 7th-11th spinal ganglion of the host and the pronephros between the 5th and 7th.¹⁾ The whole *taeniatus* trunk was reduced in length. From

¹⁾ As the trunk and tail of the *taeniatus* embryo was bent much dorsad, the position of the various organs could not be determined with regard to its own spinal ganglia.

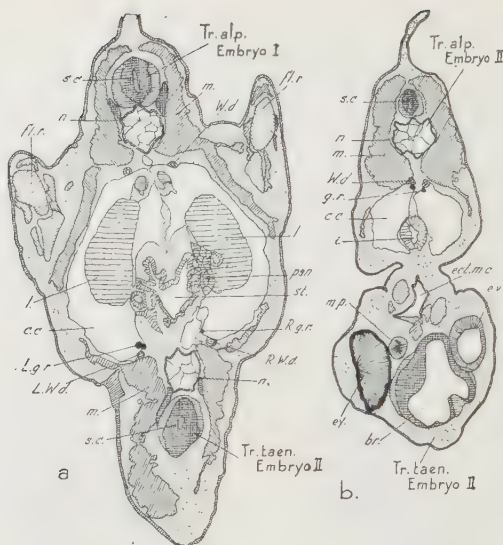


Fig. 76. Cross sections through trunk of *Tr. alpestris/taeniatus* 494. Cf. arrows in fig. 75. a) Cranial trunk region of *Tr. alpestris* (forelimb region) and middle trunk region of *Tr. taeniatus*. Development of Wolffian ducts and germ ridges of *Tr. taeniatus* embryo. b) Caudal trunk of *Tr. alpestris* and head of *Tr. taeniatus*. Enlargement $33\frac{1}{3}\times$. Ect. m.c.: ectodermal mouth cavity.

the two pronephroi Wolffian ducts had grown out in caudal direction. They run along the coelomic cavity over a length of about two segments of the host (5th-3rd sp.g.), then the coelomic cavity decreases considerably in size, and ends in the cranial part of the trunk of the host. Along the Wolffian ducts of the *taeniatus* embryo germ ridges have been formed of an entirely normal structure in the coelomic wall. They even contain some

TABLE 16

Number of animal	Number of germ cells of host	Number of germ cells of graft	Position of germ cells of graft with regard to own sp. g.	Position of germ cells of graft with regard to sp. g. of host
491/2	41	5	sp. g. 9-11	before Xth cephalic g.
493/5	57	1?	Xth cephalic g.	sp. g. 7-6
494	56	13	sp. g. 4/5-?	" " 4/5-3/4

Number of germ cells of controls. $\bar{M} = 218$. Cf. table 12, p. 133.

germ cells. These germ ridges are situated on a level with the cranial entoderm of the host, opposite the rudiments of stomach, liver and heart. ¹⁾ The first cross section (fig. 76a) clearly demonstrates these positions. The second (fig. 76b) shows the microcephalic development of the *taeniatus* head and the normal structure of the *alpestris* trunk. The number of germ cells of the partners are of interest too. Cf. table 16.

Discussion.

As the invagination of the mesoderm takes place in a cranial direction, the mesoderm of the *taeniatus* embryo at earlier developmental stages was situated opposite still more cranial parts of the *alpestris* entoderm. Therefore the formation of germ ridges in the *taeniatus* mesoderm proves that also the cranial entoderm of the host is able to bring about the general activation of the peritoneal epithelium, necessary to the formation of the germ ridges. Moreover, this experiment proves that the regional differentiation of the germ ridges (within the germ ridge region) is not determined by the entoderm, but probably by the surrounding mesodermal organs. What mesodermal organs are of importance in this respect, is still unknown, however. This difficult question is closely associated with the regional development of many other trunk organs (nephrogenous cord, nervous system, etc.).

The grafted *taeniatus* embryos and the hosts show considerably smaller numbers of germ cells than the corresponding controls. During the operation the ventral blastoporal lip of the host was cut, and the ventral mesoderm was displaced laterad. The dorsal border of the presumptive lateral plate mesoderm might, therefore, be more influenced by the axial organs than in normal animals (smaller distance between this mesoderm and the notochord). Moreover, the ventral parts of the presumptive lateral plate mesoderm of the host have come into contact with the cephalic region of the graft. In my opinion, this reduction in the germ cell number of the host has been caused by a transformation of presumptive lateral plate mesoderm into other tissues (myotomes, nephrogenous cord, cephalic mesoderm, etc.).

Judging from the experimental series 11, p. 114, germ cells might be expected in the cranial half of the "germinal region" of the graft. The few germ cells present are actually situated in the

¹⁾ The host shows a reduplication of the heart, as the fusion of its paired rudiments had been blocked by the graft.

most cranial part of the germinal region. Therefore they probably represent germ cells determined by the donor entoderm before the operation. The fact that the number of p.p. germ cells in the graft is so extremely small, indicates that, contrary to the dorso-caudal entoderm, the cranial entoderm is not able to determine the formation of the p.p. germ cells.

C) SUMMARY OF PART III.

1) The germ ridges develop from the peritoneal epithelium. In those parts of the trunk of experimental animals where the formation of the coelomic cavity has been suppressed, germ ridges are always lacking.

2) After removal of the whole entoderm at the early neurula stage germ ridges are not formed, notwithstanding the normal development of all other organs of the trunk. From this fact we are obliged to conclude that the entoderm activates the whole peritoneal epithelium in a general way, after which a second local inductive action determines the formation of the germ ridges.

3) Transplantations of the rudiments of the pronephros and Wolffian duct and of the notochord, performed at the early neurula stage, prove that the extirpation of one of these organs does not prevent the formation of the germ ridges. On the other hand, well differentiated supernumerary germ ridges may be formed along a supernumerary Wolffian duct or notochord. Both organs can induce locally the reactive peritoneum to the formation of germ ridges (double insurance). The negative results in the donors are explained by the fact that in both series one of the two organs (Wolffian duct or notochord) remained, and ensured the determination of the germ ridges.

4) Probably the germ ridge formation is also more or less dependent on the presence of the mesonephros; no experiments, however, have been made to test this assumption.

5) After the removal of the presumptive lateral plate mesoderm at the early yolk plug stage, sterile germ ridges have been formed. This proves that the p.p. germ cells are not necessary to the formation of the germ ridges. On the other hand, the sporadic observation of extra-regional gonads, formed round isolated groups of germ cells, may perhaps point to an active influence of the p.p. germ cells in the formation of the germ ridges.

6) A local influence of the myotomes or the entoderm upon

the determination of the germ ridges may be rejected, as these organs border upon a large part of the peritoneum.

7) All these facts prove that the determination of the germ ridges occurs in two phases, *viz.*, a general activation of the peritoneal epithelium by the entoderm, and a local induction by the Wolffian duct and notochord (and probably by the mesonephros and the p.p. germ cells).

8) A short series of experiments, in which the ecto-mesodermal envelope of a *taeniatus* neurula was grafted in reverse direction into the ventral side of an *alpestris* neurula has been made. It shows that germ ridges have developed in the *taeniatus* embryo in contact with the cranial entoderm of the host. This proves 1) that the cranial entoderm can activate the peritoneum in the same way as the caudal entoderm, and 2) that the cranio-caudal extent of the germ ridge region is not determined by the entoderm, but probably by the surrounding mesodermal organs. What organs these are is as yet not known.

GENERAL CONSIDERATIONS, AND PERSPECTIVES FOR FURTHER EXPERIMENTAL INVESTIGATION.

When we compare the three determination processes studied in this paper, *viz.*, those of lateral plate, germ cells and germ ridges, it is manifest that the entoderm has an important influence upon all of them. In the development of the lateral plate, the determination to splanchnopleure is dependent on the entoderm; in the formation of the germ cells, the entoderm achieves the final determination of the predetermined elements; finally, in the development of the germ ridges the entoderm activates the "reactive powers" in the peritoneum, thereby rendering it sensible to the action of the local inductors.

In the literature, inductive actions are known of the cephalic entoderm only, whereas none, or hardly any, inductive influence is ascribed to the trunk entoderm. The present investigation shows, however, that several inductive influences are exerted by this germ layer also.

These three inductions are acting, at different developmental stages, upon the same material, the lateral plate mesoderm. In the first process, the whole inner layer of the lateral plate in the trunk forms the "reaction system". In the second, the presumptive germ cells, probably scattered among the cells of the caudal part of the lateral plate, react upon the inductive influence.

Finally, in the third, the whole peritoneum of somatopleure and splanchnopleure is activated by the entoderm.

As regards the temporal relations, the following scheme seems the most likely: The determination of the germ cells takes place during the neurulation, when the presumptive lateral plate mesoderm, immediately after the invagination, passes the presumptive dorso-caudal entoderm. The determination of the splanchnopleure may last longer, *viz.*, until after the lateral plate has reached its final position. Finally, the activation of the peritoneum to germ ridge formation occurs still later, *viz.*, after the coelomic cavity has already been formed.

It may be that we have to do here with different inductive agents, but the idea suggests itself that in all these cases the same agent is at work, acting upon different reaction systems. The induction centres, however, do not entirely coincide in the three determination processes. The determination of the germ cells occurs only by the dorso-caudal entoderm, whereas the inductive actions which determine the other two, are probably exerted by the whole entoderm. It may, therefore, be presumed that the agent of the germ cell determination differs from one or both of the others. Only a further investigation of these determination processes can solve this interesting and important problem. Therefore a more detailed experimental analysis of the successive phases of these determination processes is projected for the near future.

Concerning the formation of the germ cells, it will be necessary to investigate the earliest stages of the germ cell segregation in the period from the uncleaved egg until the late neurula. Here an experimental analysis, accompanied by a histological investigation of the development of controls and experimental animals will be necessary. Under such circumstances the individual fate of the germ cells must be traced. These investigations may throw light on many interesting questions concerning the physico-chemical structure of the germ cells and the nature of the induction process.

By means of heteroplastic transplantations I hope to be able to furnish the answer to the question as to whether or not secondary generations of germ cells from somatic cells appear during the development and life of the animals. Moreover, many other problems concerning the structure, development and differentiation of the gonads may be solved by these transplantations.

The fact that the eggs of an Anure species, *Xenopus laevis* (Daud.), lend themselves admirably to experimental investigations, opens the possibility of extending these experiments also to this group of the Amphibians.

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ON THE STRUCTURE OF THE SHELLS AND
PEARLS OF TRIDACNA SQUAMOSA LAM.
AND HIPPOPUS HIPPOPUS (LINN.)

BY

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I. Introduction

The Tridacnidae have already drawn the attention of the first naturalists on account of their huge size; *Tridacna gigas* Lam. (*T. derasa* Iredale), the largest of the recent Bivalva, belongs to this family. Though the principal species, which are widely distributed in the Indo-Pacific, were described about 250 years ago, there is yet little known of their structure and mode of life, as was pointed out by VAILLANT (1865) and YONGE (1936). The morphology was studied by QUOY and GAIMARD (1835), VAILLANT (1865), GROBBEN (1898), LACAZE-DUTHIERS (1902), PEL-SENEER (1911) and others. HEDLEY (1921) published a revision of the Australian Tridacnidae. YONGE (1936) studied the mode of life, feeding, digestion and symbiosis with zooxanthellae.

A detailed description of the pearl-like concretions in *Tridacna*- and *Hippopus*-shells was given by RUMPHIUS (1705). He found them frequently in the muscles. It was only in the beginning of the present century that muscle-pearls were again described (HERDMAN 1903, RUBBEL 1912, JAMESON 1912). According to RUMPHIUS *Tridacna*-pearls are often very similar to the so-called coconut-pearls which are said to be found in the coconut. In a previous publication (REYNE 1939) it was shown that the majority of these "coconut-pearls" are certainly derived from *Tridacna* and that the existence of similar concretions in the coconut-fruit has still to be proved. As I found afterwards QUOY and GAIMARD (1835, vol. 3, p. 485) were already of the same opinion. They say with reference to *Tridacna*: "C'est aussi le lieu de parler de ces perles assez grosses, mais peu régulières, que secrète le manteau, et qui, dans plusieurs endroits de l'Inde, aux Moluques et aux Mariannes, passent pour être un produit de la végétation, et se trouver dans l'intérieur de la noix de coco".

In connection with my observations on coconut-pearls I have examined a collection of genuine *Tridacna*-pearls. It seems that beside VAILLANT's short description of a pearl of *Tridacna elongata* Lam. no other descriptions of *Tridacna*-pearls have been published since RUMPHIUS (1705). There was still another reason to study these pearls. The structure of pearls is always in accordance with the structure of the shell from which they are derived. Many detailed studies have been made of oriental and freshwater-pearls, which are produced by shells, composed of calcite- or aragonite-prisms and layers of mother-of-pearl. The *Tridacna*-shell shows a structure similar to that of the Gastropoda, the so-called crossed lamellar structure. This structure with its optical properties was described in detail by BØGGILD (1930, pp. 21-24). According to BØGGILD it is the most specialized and most common structure in Molluscs. Among 25 families of Lamellibranchia, which he examined, it was only absent in 6 families. *Unio* and *Anodonta*, the common examples of shell-structure in textbooks, with their prism- and mother-of-pearl-layers, represent exceptional cases. As far as I know, pearls from Lamellibranchia with crossed lamellae have never been examined in detail. KESSEL (1937) described a pearl of *Strombus*, a Gastropod with a typical crossed lamellar structure. It seems that this is the only detailed description of a pearl from a shell with typical crossed lamellae.

In order to obtain *Tridacna*-pearls I applied to South Celebes which is the chief centre of distribution of coconut-pearls since RUMPHIUS' time. I am greatly indebted to Dr E. J. VAN DEN BERG at Baoe Baoe (island of Boeton, S. Celebes), who, though himself a linguist, did everything in his power to collect material for me and to obtain reliable information on the occurrence of *Tridacna*-pearls. He bought several pearls from the Bugi and examined 63 living specimens of *Tridacna squamosa* and *Hippopus hippopus* himself, 8 of which contained concretions. All together 94 concretions were received of which 30 were found by Dr V. D. BERG in the shells mentioned above. Further I have received 2 *Tridacna*-pearls from Mr F. M. K. STEUP, forester in Tandjoeng Pinang (Rhio-archipelago). I have to thank Mrs W. S. S. VAN DER FEEN-VAN BENTHEM JUTTING (curator of the Zoological Museum in Amsterdam) for a shell of *Tridacna squamosa* and *Hippopus hippopus* and some material of the adductor-muscle and mantle of *T. squamosa*; further she called my attention to some publi-

cations, which I had overlooked. I am indebted to Prof. Dr C. H. EDELMAN (Wageningen) and his assistant Dr J. W. BOON for an examination with X-rays of the shell and some pearls of *Tridacna*. To Mr J. BOLMAN (Leyden) I have submitted some specimens from the Boeton-material, which are described in his monograph on pearls (BOLMAN 1941).

Tridacna squamosa Lam. and *Hippopus hippopus* (Linn.) are common on coral reefs in the Malay Archipelago. They are eaten by the natives, especially by the Badjo's or sea-gypsies. In consequence of this the shells are regularly fished in large quantities. The meat is sometimes sold on the markets (e.g. at Menado, N. Celebes), as is already stated by RUMPHIUS. The pearls found in these shells, which are described hereafter, are used by the natives as charmstones ("mustika") and the finer specimens are also sold or presented to Europeans. In the island of Boeton there is a regular trade in these pearls, which are often sold on board of the arriving steamers as coconut-pearls.¹⁾

It seems that the occurrence of pearls or concretions in *Tridacna*- and *Hippopus*-shells is more or less local. RUMPHIUS says that they are scarce around Amboyna, but more abundant near Macassar and New Guinea. According to Drs J. VERWEY and J. D. F. HARDENBERG *T. squamosa* is common in the Bay of Batavia and also used as food, but concretions are unknown there. Prof. Dr C. M. YONGE (Bristol), who spent a considerable time on the Great Barrier Reef in Australia, informed me that he is not familiar with these pearls, though he has opened many hundreds of *Tridacna* and *Hippopus*. It seems that they are unknown in the Solomon- and Fiji-Islands, as Mr R. A. LEVER, govt. entomologist in these islands, informed me. JAMESON (1902) mentions the local occurrence of pearls of *Tridacna gigas* Lam. in Torres Straits and around New Guinea, which he thought to be due to some pathological condition, e.g. the presence of Trematodes which he found in *Tridacna*- and *Hippopus*-shells. In the Malay Archipelago *Tridacna*-pearls are known from S. Celebes, New Guinea and adjacent islands, further from the Banggai- and Rhio-archipelago. The following species of *Tridacna* are reported from the Dutch East Indies by VON MARTENS (1902) and PRASHAD (1932): *T. gigas* Lam., *T. squamosa* Lam., *T.*

¹⁾ Other false pearls, sold in Boeton, are certain black pearls made from the shell of *Pinna nigrina* Lam. (W. J. SCHMIDT-Z. f. Morph. u. Oekol., 25, 1932, pp. 284-287) or *Malleus* (J. BOLMAN 1941, pp. 51-54; 143, 145).

crocea Lam. (= *cumingii* Reeve), *T. serrifera* Lam. and *T. elongata* Lam. All these species (except *T. serrifera*) are reported as being used as food by the natives; *T. mutica* Lam. is eaten in Guam according to QUOY and GAIMARD. Pearls are known from *T. gigas*, *T. squamosa* and *T. elongata* and if the identifications of RUMPHIUS' species by VON MARTENS (1902) are correct, also from *T. crocea* and *T. serrifera*.

The structure of pearls and shells was studied by grinding surfaces in different directions, by examination of thin splinters and by dissolving the objects in acids. Some pearls have been watched during grinding and dissolving from beginning to end. Others have been examined by means of X-rays. During the war-action at Wageningen in 1944 and 1945 the collection was lost, so that I could not study the objects by thin sections, which had still to be made by an expert in this technique. For the same reason I am unable to offer photographs of some pearls and sections as I intended to do; the textfigures were drawn after sketches in my notebooks.

2. The shell-structure of *Tridacna* and *Hippopus*

The structure of the *Tridacna*-shell was recently described by BØGGILD (1930) and BOLMAN (1941). BØGGILD gives a short description of a *Tridacna* spec. (species not mentioned, no figure): "The shell of a *Tridacna* sp. (recent) has an especially simple construction consisting only of one layer of crossed lamellae oriented concentrically (around the umbo-R.). The lamellae are, as usual, almost vertical in the lower part and gradually strongly reclined upwards. They are rather coarse (ca 0.05 mm) and regular, the sections parallel to the surface showing only faint traces of the common rhomboidal figures."

BOLMAN's fig. 41 shows microphotographs of sections through the shell of *T. elongata*. Reference to these figures is made in my description of the shell-structure of *T. squamosa* which follows below.

A young specimen of *T. squamosa* was examined; dimensions of shell 7×5 cm, weight about 25 grams, specific gravity 2.77 (in *T. elongata* according to BOLMAN 2.7656, according to VAILLANT 2.75). The carbonate of lime consists of aragonite as was shown by an examination with X-rays (Debije-Scherrer powderdiagram); the material was taken from the hinge and

the edge of the shell opposite to it and examined by Dr J. W. BOON (Wageningen).

The outer surface of the shell is covered by a brown organic membrane, which does not dissolve in acids and stains vigorously with eosin. It is about 0.005 mm thick and seems to be only present in young specimens. The statement of ZITTEL (1910), YONGE (1936) and others that a periostracum in the Tridacnidae (resp. *T. crocea*) is absent, refers probably to older specimens.

On the inner surface of the shell white veins are seen, which run in the direction of the growth-lines, parallel to the distal edge of the shell. Especially where the shell is bent and on the impressions of adductor- and mantle-muscles, where the surface is very smooth, the veins are clearly visible with a pocket-lens ($8\times$). These veins intercommunicate with each other and are caused by the edges of the crossed lamellae which are placed vertically to the inner surface of the shell. In other shells with crossed lamellar structure like *Cardium edule* L., *C. setosum* Riss., *C. subrugosum* Sow. etc. and in several of the marine Gastropoda the same veins are present.

The *Tridacna*-shell is almost wholly composed of crossed lamellae. According to BØGGILD these lamellae are built up of smaller lamellae of the second order as shown in fig. 1. These lamellae II cross each other in adjacent lamellae I at an angle of about 90° , so that sections parallel to the lamellae I show fibre-crossing (cf. BOLMAN, fig. 41 *bb*). The lamellae II meet the inner surface of the shell at an angle of about 45° . As the course in adjacent lamellae differs about 90° , the lamellae 1, 3, 5, 7 have their fibres oriented in the same direction. When these lamellae reflect the light, they will appear white and the lamellae 2, 4, 6, 8 dark. After turning the section 180° lamellae 1, 3, 5, 7 will appear dark and lamellae 2, 4, 6, 8 white. The white venation (fig. 4) on the inner surface of the shell and in horizontal sections (i.e., parallel to the surface) is caused by different fibre-course in adjacent lamellae. The same applies to radial sections (i.e., through the umbo and vertical to the surface and growth-lines). Tangential sections (i.e., vertical to the surface and along the tangent of the growth-lines) will show a crossing of the fibres, at least in the lower part near the inner surface where the section is parallel to the lamellae.

In a radial section of the shell of *T. squamosa* the lamellae I, alternately white and dark, are clearly visible at low magnifi-

cation (6-10 $\times$). In the lower part of the section they stand vertically to the inner surface of the shell; in the upper part they are bent umbo-ward and fade away. Near the outer surface the lamellae are almost parallel to this plane and all their fibres have a radial course, as is shown by horizontal sections near the outer surface. The width of the lamellae near the inner surface varies, according to many measurements, from 0.03-0.04 mm (average 0.033 mm). When a radial section is turned 180°, the white veins become dark and the dark veins white (fig. 2). In the nail-like projections of the shell the same structure is found; the average width of the lamellae was 0.038 mm. The white and dark veins (edges of lamellae I) are most conspicuous in incident light, parallel to the lamellae. As the lamellae are rather narrow in the direction of the growth-lines and united with their edges to other lamellae, they show slight branching in radial sections and a more or less distinct reticular design in horizontal sections (fig. 4).

Besides the dark and white veins, caused by the crossed lamellar structure, dark growth-layers are seen in radial sections; their course is perpendicular to the crossed lamellae and near the inner side parallel to the surface of the shell. The lamellae I are placed vertically and the dark growth-layers parallel to the secreting surface of the mantle. These dark growth-layers are clearly shown in BOLMAN's fig. 41a, a radial section through an older shell of *T. elongata*. A striation, perpendicular to the growth-layers, is shown in his fig. 41aa; these streaks are the edges of the crossed lamellae. The dark growth-layers are white in incident and brown greenish in transmitted light. They are caused by minute granules which are apparently of an organic nature. As the granules gradually fade away after slow solution in acids without moving, they seem to be neither gas-bubbles nor fluid- or conchyolin-drops. The dimensions are 0.001 mm or less. These granules, which are also present in other shells, are generally interpreted as conchyolin-globules or gas-bubbles. I am more inclined to take them for a pigment formed during the intermittant secretion of the mantle, but the true nature of these granules has still to be determined. They do not stain with eosin or hematoxylin and are soluble in acids, probably also in alkalis. Where they are thickly crowded the solution of the calcareous matter is slowed down by their presence; the same was often seen in pearls which contain a large proportion of organic

matter. Some authors interpret these dark growth-layers on the analogy of LIESEGANG-rings (cf. HEDGES 1932); the structure of the nuclei in pearl No. 17, described hereafter, can hardly be explained by intermittent secretion.

The fibre-crossing in tangential sections can be easily demonstrated in tangential splinters which are slowly corroded by acids. Such splinters or scales can be split off without difficulty with a knife, due to the orientation of the lamellae I. The corroded edges of these splinters show a fringe of crossed fibres (fig. 3). After the solution is completed a jelly-like organic matrix remains, which does not stain (or only very weakly) with eosin. The proportion of organic matter in shells with crossed lamellae is always very small, about 2-3 per cent or less (BÜTSCHLI 1908); in *Tridacna* the amount is 2.25-2.26 per cent according to VAILLANT (1865). In some layers at the base of the nail-like projections, where the dark granules were very thickly crowded, a fine prismatic structure was found. These prisms, which are only slightly thicker than the fibres of the crossed lamellae (measuring about 0.001 mm), are torn off during solution, while the crossed fibres disappear slowly without being detached (fig. 3). The dark growth-layers parallel to the inner surface of the shell are also visible in tangential sections.

Horizontal sections (parallel to the surface) show a more or less regular network of white veins as were described above for the inner surface of the shell. Just as in radial sections these veins are caused by the edges of the lamellae I.

VAILLANT's description of "traînées parallèles plus ou moins irrégulièrement anastomisées les unes avec les autres, composées d'une multitude de petites stries en chevrons . . . qui sont parfois excessivement difficiles à distinguer" seems to refer to a radial, somewhat oblique section; the width of the "traînées", according to his fig. 8 is 0.04 mm and not 0.004 as stated in the text. CARPENTER (1847, p. 100) says that the principal feature of the shell of the Tridacnidae is its "corrugated structure" and refers to his fig. 43 (CARPENTER 1844) which shows a horizontal section of a *Lima*-shell with anastomosing veins.

A diagram of the shell-structure of *T. squamosa* is given in fig. 4.

On the muscle-impressions of *T. squamosa* the hypostracum-layers ("durchsichtige Substanz", "helle Schicht" of German authors) seem to be absent or extremely thin and transparent,

as the white veins (edges of lamellae I) are passing without any interruption over the muscle-impressions. Neither were hypostracum-layers seen in cross sections (ground surfaces; not examined in thin sections). In pearl-oysters and other nacreous shells the hypostracum consists of a modification of nacre (SCHMIDT 1924). EHRENBAUM (1885) has described a hypostracum on the muscle-impressions of *Cardium edule*, viz., a prismatic structure with growth-layers parallel to the surface. This is the only description, as far as I know, of a hypostracum in shells with crossed lamellae. In *Cardium edule* I found the white venation of the inner surface of the shell interrupted on the muscle-impressions of the adductores, but on the mantle-line it is clearly visible as in *Tridacna* and *Hippopus*. The muscle-impressions of the adductores show densely crowded growth-layers parallel to the surface and fibres perpendicular to them, but I failed to find a distinct prismatic structure. In *Tridacna* and *Hippopus* the course of the fibres on the muscle-impressions is probably vertical to the surface, but dark growth-layers are absent, so that the edges of the crossed lamellae are clearly seen through the transparent mass.

In *Tridacna squamosa* a distinct prismatic structure is found in the conchyolin-layer below the ligament, which is impregnated by CaCO_3 ; it is already visible at low magnification ($10\times$). After corrosion by acetic acid the prisms show a fine cross striation. The distance of the stripes is about 0.0015 mm; at intervals of 0.01 mm thicker stripes are present. After all calcareous matter has been solved a mass of granular threads remains, which are 0.005–0.010 mm thick. The same prismatic layer is found in shells of *Hippopus hippopus* (L.) and *Cardium edule* L. Some parts of the hinge have also a more or less distinct prismatic structure; it is more clearly developed in the hinge of *Hippopus*.

It seems that the structure of the *Hippopus*-shell has never been described or figured; the specific gravity was determined by KELLY at 2.81 (BÜTSCHLI 1908). I have examined a shell of 17×11 cm, weighing about 400 grams. The microscopic structure is very similar to that of *Tridacna squamosa*, but the orientation of the lamellae I is somewhat modified by the development of strong ribs on the distal part of the shell. The width of the lamellae in radial sections of the distal edge of the shell varied from 0.029–0.033 mm. The muscle-impressions and the prismatic

layer near the hinge have the same structure as in *Tridacna squamosa*. A tooth-like excrescence with peculiar structures, found on the inner side of a *Hippopus*-shell, is described in § 3 under No. 19.

3. *Tridacna*- and *Hippopus*-pearls

According to RUMPHIUS (1705) *Tridacna*-pearls are found in the "tendo" and surrounding "spondylum" which are the edible parts of the Mollusc. From his further description it is quite evident that the name "tendo" is applied to the large adductor-muscle. The name "spondylum", described as a hard meat surrounding the "tendo" like a round disc, seems to refer to the mantle-muscles. RUMPHIUS says that in a *Tridacna*-species of the Lucipara Islands (*T. serrifera* according to VON MARTENS) the concretions are sometimes present in such a quantity that the whole "tendo" seems to be composed of these stones. The concretions were only found in 10 per cent of the squamous *Tridacna* and only in those larger than a foot.—Among 63 specimens of *T. squamosa* and *Hippopus hippopus* (measuring $20-24 \times 15-16.5$ cm) from Boeton and Moena, examined by Dr E. J. VAN DEN BERG, 8 or nearly 13 per cent contained concretions. — According to RUMPHIUS the *Tridacna*-stones are very similar to coconut-pearls. He had never found coconut-pearls himself, but on many occasions stones in *Tridacna*-shells. The latter are commonly irregular, angular and yellowish. Sometimes they are aggregates from which separate parts may split off ("breeding pearls"). RUMPHIUS had even found *Tridacna*-stones in the smoked meat ("bia den-deng"). The finer specimens, which are white and regularly shaped, lie loosely in the meat and are generally not larger than a pea; one of the ends is often smooth and transparent like agate. The angular specimens are sometimes bottle- or retort-shaped and fastened to the shell. All these pearls "boil" in lemon-sap and vinegar. It seems that RUMPHIUS was familiar with concretions of *Tridacna gigas*, *squamosa*, *crocea*, *serrifera* and *Hippopus hippopus*; identifications of R.'s Molluscs are given by VON MARTENS (1902).

VAILLANT (1865) describes very shortly a pearl-like concretion of *Tridacna elongata*. "Leur structure est celle de la coquille, seulement la matière paraît encore moins nettement organisée, les cellules (the network of white veins; R.) y sont peu visibles,

on y distingue cependant les fibres en chevrons, parfois la masse se divise aussi en prismes". According to VAILLANT these concretions are rather often found in the interior part of the mantle, which lies within the line of attachment of the mantle-muscles.

More details are given by BACON (1861) in his description of a "coconut-pearl" from Singapore. The surface was white, slightly mottled; faint undulate markings were seen within. Sections showed numerous concentric lamellae. The centre was occupied by a semi-transparent mass; a foreign nucleus was absent. The general mass of the pearl was made up of "radiating bands of crystalline fibres, inclined at different angles in contiguous bands". In the outer layers the crystalline structure was sharply marked by rhomboidal cleavage.

KORSCHOLT (1913) gives a short note on irregular *Tridacna*-pearls, "die zwar auch konzentrisch geschichteten, aber grösstenteils aus einer kristallinischen Kalkmasse bestehenden und in ihrer Struktur sehr unregelmässig erscheinenden Perlen von *Tridacna*".

Dr FRIEDRICH WEBER (Lugano) informed me, that during his residence in S. Celebes he bought over a hundred *Tridacna*-pearls and also some coconut-pearls in Boeton and the Moluccas. After examining the microscopical structure of the latter, he found that they were identical with *Tridacna*-pearls. In 1932, when I was connected with the Coconut Experiment Station in Menado (N. Celebes), Dr W. asked me to collect genuine coconut-pearls for him and gave me the following description of his *Tridacna*-pearls. "They are spherical, ovoid or pear-shaped, in many cases also thick lenticular or semi-globular or semi-lenticular (plano-convex). The surface is generally smooth and shining, the colour porcellaneous, milkwhite, sometimes greyish white or light bluish white and in this case glazy. With a good pocket-lens (6-10 $\times$) one can see thin fibrous lamellae in the interior, which usually show a curved, spiral course and converge at the poles. At variance with the concentric structure of common pearls the *Tridacna*-pearl is built up of radial lamellae and has a bipolar structure. This characteristic is at the same time the cause of a second one, viz., a ring-shaped cat's-eye lustre around one or both poles, which in moving the pearl changes its place like the air-bubble of a water-level. When the lamellae are somewhat broader this lustre shows a soft bluish but never a nacreous shine. This phenomenon, caused by thin radial lamellae

or fibres of aragonite, which run meridionally, is a characteristic of the *Tridacna*-pearl. A thin equatorial section shows concentric rings in the interior and a radial fibrous or stalk-like structure in the outer parts." Dr W., being a geologist, studied his *Tridacna*-pearls in thin sections, but to my knowledge his research has never been published, as he intended to do. It seems that he bought only regularly shaped *Tridacna*-pearls; the irregular specimens, which are far more common, are not offered for sale.

BOLMAN (1941) described the following objects of my *Tridacna*-material from Boeton in his monograph (pp. 134-137, microphotographs on pl. 34).

1. A semi-globular, half-transparent, veined and bipolar pearl, bought from Buginese merchants. Weight 661.5 mg. Specific gravity 2.8785. Two sections (magn. $16\times$), parallel to the basal plane of the pearl and perpendicular to it, are shown in B.'s fig. 1a and 1c. I cannot agree with B.'s conclusion that this pearl is composed of calcite for the following reasons. A specific gravity of 2.8785 points to aragonite (S.G. 2.9) and not to calcite (S.G. 2.7). According to a review of all previous determinations of the specific gravity of shells by BÜTSCHLI (1908), one can safely accept that the calcareous matter is aragonite, when the S.G. is over 2.75 ("beträgt das S.G. 2.75 und darüber, so ist Aragonit fast sicher"). The S.G. of the CaCO_3 in the shell is always less than that of the pure mineral on account of the admixture of organic substance (conchyolin S.G. = 1.34). The "uniaxial image", shown in B.'s fig. 1b, is a spherulite-cross, from which it is impossible to derive whether the mineral is uniaxial calcite or biaxial aragonite; the same applies to his fig. 3a and 3b. The chemical reaction, mentioned by BOLMAN, appears to be unreliable (see No. 4). Only aragonite has been found in my *Tridacna*-material from Boeton; the same applies to the *Tridacna*-and coconut-pearls of Dr FR. WEBER and the coconut-pearl examined by myself (REYNE 1939). A second semi-globular pearl in my collection, similar to the object examined by BOLMAN, is according to a Röntgen-diagram certainly aragonite. Furthermore the objects were sold as being found in the "moraboe", i.e. *Tridacna squamosa*. In my opinion BOLMAN's fig. 1c shows a close resemblance to the structure of concretions taken from *Tridacna*-shells.

2. Two irregular concretions, bought from the Bugi, who asserted that these objects had been taken from "moraboe"-shells.

As the "moraboe" is common and often eaten and as the objects are worthless for common trade, there is no reason to doubt their statement (only 15 cents were paid for about 30 of these concretions). Weight 937 and 1067.4 mg, specific gravity 2.8610 and 2.8663. According to B. laminae and fibres are in accordance with the structure of a *Tridacna*-shell (cf. B.'s fig. 2a, 2b, 3a, 3b).

3. An irregular concretion, taken by Dr VAN DEN BERG from the adductor-muscle of *Tridacna squamosa*. Dimensions 7.5×6.8 mm; weight 342.1 mg; specific gravity 2.8652; fig. 4a, b, c (BOLMAN, pl. 34). Fig. 4b shows a zigzag structure (fibre crossing. R.) between crossed nicols, fig. 4c, a concentric structure with radial fibres, which is also present in fig. 2.

4. A large "mustika", registered as V.K. 189/1 in the Indian Museum at Amsterdam, which is probably a *Tridacna*-pearl, was also described and figured by BOLMAN (p. 142). Diameter 19.6 mm, height 17 mm, weight 9439 mg, specific gravity 2.8224. The pearl is globular and shows a cavity at one of the poles, where it was apparently attached to the shell. Near the other pole some faint white veins are seen as in *Tridacna*-pearls (R.). B. says that the calcareous matter is calcite according to chemical reaction. Its specific gravity, however, points to aragonite. A re-examination by Dr BOON by means of X-rays of some material, taken from the cavity, showed that this pearl is without doubt composed of aragonite (fig. 28).

With regard to the outer appearance of *Tridacna*-pearls I have nothing to add to Dr WEBER's description of regularly shaped specimens. With the exception of semi-lenticular forms I have seen all the shapes which he mentions. Only in a few specimens a ring-shaped cat's-eye lustre was observed, as described by Dr WEBER and RUMPHIUS; in my own collection there was only one small pearl which showed this phenomenon. Few regularly shaped specimens were present among the material, bought by Dr VAN DEN BERG in Boeton, since little money was spent on it; regular *Tridacna*-pearls with a beautiful lustre are rather expensive. By far the greater part were irregular concretions; all 30 specimens, which Dr VAN DEN BERG collected himself, belong to this class with one or two exceptions. The regular pearls with white veins are generally not larger than a pea or bean; very few are as large as a cherry. Among 42 irregular pearls, bought in Boeton, the size varied between 5 and 18 mm; the total weight of these objects was 28.51 grams, their volume about 9.5 cc (only

roughly measured with a measuring glass). A photograph of a gigantic concretion, weighing 14 lbs 1 oz. (dimensions 9.5×5.5 inch), taken from a *Tridacna gigas*, by which a native diver had been killed, was published by COBB (1939). Of the 42 gravel-like *Tridacna*-concretions mentioned above, 10 showed a distinct place of attachment, 9 others were probably also attached to the shell or other concretions; in 2 specimens there were 2 stalk-like excrescences, opposite to each other, by which they had been fastened to some object. Of the same lot 17 specimens showed internal cracks, 23 broad shallow grooves, parallel to each other, 5 were very warty, 5 more or less mottled with white spots, 2 specimens had crest-like excrescences (fig. 5). The concretions were generally opaque white, a few more or less glazy and bluish. One specimen showed a small pearl embedded in a larger one; the same was observed in one of the concretions, found by Dr VAN DEN BERG in a *Tridacna squamosa* (fig. 6). One specimen was irregularly grooved so that its surface had a brain-like appearance.

The two *Tridacna*-concretions, bought in the Rhio-archipelago by Mr F. W. K. STEUP, were irregular and warty; they showed shallow parallel grooves.

The outer appearance of those concretions, which Dr VAN DEN BERG collected himself from *Tridacna squamosa*, are described separately, as their origin is known with certainty. He examined 23 specimens of *T. squamosa* and *Hippopus* from Poeloe Makassar in the Bay of Bae Bae (island of Boeton). Only in one *Hippopus* a small concretion was found and in another one a tooth-like excrescence. Further he examined 40 living shells of *T. squamosa* and *Hippopus* from Mawasangka (island of Moena). In 2 *Hippopus* and in 5 *T. squamosa* concretions were found. Those of *T. squamosa* are described below.

1. Largest diameter 5.5 mm. Thick lenticular, almost globular. One half dull white, the other half smooth, more bluish, and covered with remains of organic matter. On the edge of the lens some white veins and spots are visible. Taken from the adductor-muscle.

2. Diameter 4 mm. Ellipsoid, somewhat bumpy, largely covered by organic matter. On one spot some coarse veins. Taken from the adductor-muscle.

3. Six small concretions (1.5–3 mm), taken from the adductor-muscle of one shell; two of them covered with organic substance.

4. Diameter 4.5 mm. Ellipsoid, dull yellowish. Not covered by organic matter. Found outside of the adductor-muscle.

5. Seventeen concretions collected from one single shell; 14 of them were found in the adductor-muscle, 3 others in a cavity with elevated rim on the inner side of the shell, near the hinge (distance 2 cm). Among the adductor-concretions the largest is 11 mm; it is dull white and shows a lobed brain-like surface. A second one, measuring 7 mm, is very irregular and provided with a short, brown, hollow stalk of attachment. Four others are small (2-3 mm) and rather regularly shaped. One is pyramidal (4 mm) and covered by organic matter in which a separate concretion was found, described hereafter under No. 11. One specimen of 6 mm showed 2 smaller concretions embedded in its mass and 3 separate specimens attached to it by organic matter (fig. 6). Another specimen had also a separate smaller concretion attached by a strand of organic matter (fig. 7). The 3 concretions, which were found in a cavity of the shell, measured 8, 8, and 6 mm. They were irregularly shaped and dull white; one specimen showed a place of attachment with a cavity. One of these adductor-concretions was described and figured by BOLMAN (pl. 34 fig. 4 *a*, *b*, *c*).

All concretions, collected from *Tridacna squamosa*, are similar to the gravel-like specimens, which were bought in Boeton and according to the sellers produced by the "moraboe" (*Tridacna squamosa*). Only few specimens show some white spots or indistinct veins. Dr VAN DEN BERG states that the majority of the concretions was found in the adductor-muscle on the side of the hinge (where the muscle retires from the shell-surface; R.). According to the natives the concretions near the hinge are the whitest, while those at some distance are more yellowish. QUOY and GAIMARD (1835) and VAILLANT (1865) state that the concretions are formed in the mantle. RUMPHIUS (1705) and Dr VAN DEN BERG found them principally in the muscles.

The *Tridacna*-pearls, bought and collected in Boeton, were studied by grinding and dissolving some specimens from beginning to end, while the change in appearance was continually watched. Details of ground surfaces are often more conspicuous after etching with dilute acid. Other specimens were broken and the surfaces and splinters studied; some parts were ground and others dissolved in acids. Some concretions and pearls were examined by means of X-rays by Dr BOON. BOLMAN's description

of some specimens from the same lot has been referred to above (p. 216).

As the structure is rather variable, it is separately described for various pearls.

A. *Tridacna*-concretions, bought in Boeton, according to the sellers found in "moraboe" (*Tridacna squamosa*). Two of these concretions were already described and figured by BOLMAN (1941, pl. 34, fig. 1 and 2).

1. A dull white specimen without veins. Ground from beginning to end; ground surface perpendicular to the shallow grooves. The centre is occupied by a semi-transparent mass with some cracks and a faint radial striation. The outer parts show radial fibres and bands (resembling somewhat the figure of a radial section of the *Tridacna*-shell) and numerous dark growth-layers parallel to the surface, which are white in incident and dark in transmitted light.

2. A glazy, bluish specimen with some cracks was crushed with a hammer. The whole pearl consists of prisms, arranged according to the radii. On ground surfaces, perpendicular to the surface of the pearl, many concentric growth-layers are visible. In addition to this the prisms show a fine cross-striation, especially after a slight corrosion by dilute acid; distance of the stripes 0.003-0.005 mm, of the growth layers 0.03-0.05, sometimes 0.1 mm. After solution of all calcareous matter the organic matrix showed a radial structure.

3. One specimen, similar to No. 1, was slowly dissolved in dilute acetic acid. After decalcifying two days the remaining organic matter on the surface showed in one place two rows of radial spines of a more dense substance, arranged parallel to adjacent white veins (fig. 8). White veins on the surface were seen in different stages of solution, but only locally. After solution was completed some remains of foreign organisms were found in the organic matrix, viz. a few small eggs or cysts and their skins and some other particles which could not be identified.

4. A large rod-like specimen with rounded ends was broken across with nippers. The surface of the fracture was like that of a piece of bone-glue. Some thin scales, which had been split off, showed a bumpy surface and after grinding and etching a mosaic-structure with indistinct fibres. The edges corroded by acid were fairly straight; the organic matrix was dense and granular. In some places, especially in the outer parts of the pearl, the fibres

had more clearly developed. Sometimes they were V-shaped and arranged in piles (chevron-structure); crossing fibres were also observed. In this case the corroded edges were fringe-like and the remaining organic substance was less dense. Ground surfaces, parallel to the superficial grooves and perpendicular to the axis of the concretion, showed 2 or 3 systems of concentric growth-layers, arranged around different centres. Faint radial fibres, perpendicular to the growth-layers, were only locally developed, other parts seemed to be amorphous or granular. This concretion is probably an aggregate.

5. A gravel-like specimen, dissolved in dilute acetic acid. After decalcifying 3 days, 2 spine-like projections of a denser substance were seen in the organic matrix, similar to those of No. 3. The undissolved part had some sharp angles and 2 smaller concretions imbedded in its mass, which detached themselves on the 4th day. These separated parts showed in the last stages of solution a fibrous zigzag-structure; the remaining body had partly a zigzag, partly a chevron structure. Apparently 2 angular concretions had been united and overgrown by secondary layers, so that the shape became more and more rounded. The granular organic matrix of the smaller concretions was stained strongly by eosin. A structure-like pavement-epithelium with some circumvallated pores (also present in No. 4) was found in one place and taken for a rest of the mantle-epithelium, but after comparing it with the mantle of *Tridacna squamosa* I doubt whether this interpretation is correct.

Just as in other concretions, which have been dissolved in acid, the organic matter was unequally distributed. It was generally membranous and fine granular, but in some parts like a dense, tough jelly; grains and small lumps of denser substance were also found on the membranes. A dense organic matrix usually stains with eosin, at variance with the less dense organic matter, left after solution of fibrous calcareous substance.

6. A glazy pearl, measuring 9×12 mm, with many cracks, was broken by means of nippers. The surface of the fracture showed an unobscured radial fibrous structure, which became clearly visible after application of acid. After grinding the surface on a hone, faint concentric growth-layers arranged around the centre were visible. Some of the splinters showed a coarse fibrous or fine prismatic structure, others a mosaic-structure (fig. 9). After corrosion by acid the pearl was covered by a mass of small

grit, as the calcareous matter was decomposed in small bundles of coarse fibres or minute rectangular prisms, much shorter than the fibres. Organic matter was scanty.

7. A small yellow pearl (diameter 5 mm) was broken by nippers. The yellow colour is caused by organic matter on the surface; the interior is white. The surface of fracture shows radial prisms with a fine cross-striation. After grinding and etching one half of the pearl many concentric growth-layers became visible, grouped around the centre, which contained a small cavity lined with organic matter and provided with radiating cracks. The organic matter on the surface and in the central cavity stained vigorously in eosin. Along the prisms and also across them a few red lines were seen, which were probably caused by organic matter (magnification $65\times$). The organic matrix, left after decalcification, was rather dense, granular and sometimes thready, but it did not stain with eosin. The corroded edge of separated prisms was straight and not fibrous. The prisms had a tendency to split across.

8. A glazy, bluish specimen, was easily crushed by a hammer. An examination by means of X-rays (Debije-Scherrer diagram) showed that this concretion was composed of aragonite. The shape of the pearl was irregular, ellipsoid; dimensions 8×12 mm, weight 0.9 grams. Two white spots were seen within. After breaking the pearl, it became evident that these spots were caused by cavities lined with a white yellowish mass of globules. These globules were spherulites with a dense organic matrix; the largest were about 0.15 mm. Especially after application of dilute acid the spherulitic structure became clearly visible. Some globules were almost wholly composed of organic matter with the calcareous spherulite developed only in one sector. One place of the cavity contained bundles of minute prisms, which after decalcification left brown rods of organic substance. The spherulites showed a radial structure after solution of the calcareous matter, in some cases also concentric layers. In several instances a small cavity (or strongly refractive substance?) was seen in the centre of the globules. (Cf. No. 19, figs. 23 and 24).

The organic matrix of the substance that lined the cavity was membranous and contained, besides the remains of the spherulites (often crowded in aggregates), numerous grains which were strongly stained by eosin (fig. 10). The membrane itself was not stainable by eosin and rather brittle as it did tear easily. In some

places its structure was more or less fibrous. A few circumvallated pores, as mentioned under No. 5, were observed in these membranes.

On the surface of fracture an un conspicuous fibrous or prismatic structure was seen, radiating from an eccentric point. In the grit, produced by breaking the pearl, indistinct prisms were found with feathery or undulating lines. Solution in acetic acid occurred very slowly; the organic substance was dense and stainable by eosin. Other particles showed partially developed spherulites with the fibres greatly prolonged at one side; the dense organic matter near the spherulites contained eosinophilous grains as mentioned above; in the fibrous part these grains were lacking, the organic substance being less dense. Where the organic matter was most dense a columnar structure was found (fig. 11) besides spherulites.

Only few growth-layers were present, surrounding the cavity; they were formed by similar granules as found in the shell. A dense organic matrix with peculiar structures and the absence of a distinct prismatic or fibrous structure is remarkable in this pearl at variance with the structure of the glazy specimens, described under No. 2 and 6.

9. An opaque white concretion, difficult to crush with a hammer, consists of aragonite according to a Röntgen-diagram, made by Dr BOON. Its surface shows on some places coarse white veins which are continued on the surface of fracture (fig. 12). After turning the object 180° the effect white-dark is reversed just as in the crossed lamellar structure of the shell, which in both cases is apparently due to a crossing of fibres in adjacent parts. After solution of the CaCO_3 a jelly-like organic matrix, similar to that of the shell, remains.

A small cavity was present, in which a few spherulites were found.

Several particles of grit were examined. Most of them were amorphous, slightly granular, and contained a dense organic matrix. In some of these particles a mosaic-structure was seen after corrosion by acids. Other particles showed a fibrous structure, sometimes undulate, mostly straight with crossing fibres; in this case the organic matrix was less dense and did not stain with eosin. In a few particles dark growth-layers, composed of the same granules as found in the shell, were present with crystal-fibres crossing them at an angle of about 45° . A prismatic and a

chevron-structure was also found. A flat white opaque portion of this pearl was almost wholly composed of conchyolin which was only slightly calcified. It appears that all structures from repair-conchyolin (cf. JAMESON 1912) and spherulites to crossed lamellae were represented in this concretion.

10. A gravel-like concretion with a place of attachment and opposite to it a glazy spherical pearl (fig. 13). Some material was bored from the place of attachment. The substance was rather soft and contained a large portion of organic material consisting of lumps and granules. In some places a fibrous or a columnar structure was seen in the organic matrix; a few circumvallated pores (see fig. 11) were also present. The structure of the calcareous matter was mainly prismatic; the prisms were often arranged in the shape of a fan or cone and most of them showed a fine cross-striation; the distance of the lines was 0.005 mm or less. This concretion is certainly an aggregate; two glazy pearls are embedded in its mass (fig. 13 *c* and *d*).

B. Concretions collected by Dr E. J. VAN DEN BERG from *Tridacna squamosa* found near Mawasangka (island of Moena, S. Celebes). One of these concretions is described and figured by BOLMAN (1941, pl. 34 fig. *a, b, c*).

11. A small accessory pearl, measuring about 1.0×1.7 mm, was dissolved in acid. It was connected by a strand of cross-striped muscle-fibres to a larger pyramidal concretion of 4 mm length. The muscle-fibres lay in a shallow depression of the larger concretion and were partly incorporated in the accessory pearl.

The depression of the larger concretion showed a prismatic structure; the prisms lay parallel to the surface and their ends were projecting like conic warts along the edge of the depression at the blunt end of the pearl.

The accessory pearl was flat and showed conic warts on its surface, where it was in contact with the mother-pearl. Near the end, where the muscle-fibres entered, there was a spherical portion, which was more or less transparent and provided with a star-like figure, probably caused by cracks in the interior. The rest was very opaque and remained so during solution. The calcareous matter seemed to be amorphous and granular. Only in the last stages of solution a number of minute prisms was seen. The organic remains were largely composed of cross-striped muscle-fibres, in some places mixed with tendinous tissue. The

muscle-fibres were partly covered by an epithelium (cells 0.005–0.010 mm), in which a tubular gland was found. After the tissue was stained by eosin and hematoxylin, distinct nuclei were only found in the tubular gland, not in the muscle-fibres and epithelium.

As little is known about the histology of *Tridacna squamosa* it is difficult to interpret the organic remains which are found in *Tridacna*-concretions. It is, however, quite clear that the present pearl was formed and found within the muscles, as reported by Dr VAN DEN BERG.

A portion of the mantle of a *Tridacna squamosa*, during many years preserved in alcohol, was examined, but no clear image of the epithelium could be obtained. The mantle consists almost wholly of connective tissue which is extremely tough and difficult to cut. A few cross-striped muscle-fibres were found in it, furthermore numerous corpuscles, stainable by eosin and hematoxylin, and some yellow grains and lumps which did not stain.

Some of the organisms and organic remains, found on the mantle-surface, were also obtained from the concretions which had been bought in Boeton.

A piece of adductor-muscle, cut from the place of insertion, was examined, but it was wholly composed of tendinous tissue without any muscle-fibres.

12. A small accessory pearl of about 1 mm (*c* in fig. 6) was dissolved in acetic acid. As the solution went on very slowly, it was continued in HCl conc., but even then it took an hour before all calcareous matter was dissolved. The pearl was fairly transparent and showed a distinct prismatic structure; some of the prisms were slightly S-shaped. Only in one place a fine cross-striation was observed on the prisms. A dense organic matrix, almost wholly amorphous, was left; only in 2 places a columnar structure was found. Muscular or tendinous tissue was not present, though the pearl was found in the adductor-muscle. The organic matrix was only slightly stained by eosin.

A membrane which covered the pearl stained strongly in eosin. Almost all concretions, collected from *Tridacna*-muscles, showed such membranes adhering to the surface; muscular or tendinous tissue was seldom found in them. These membranes were mainly amorphous and showed only locally a fibrous structure. Sometimes yellow lumps or grains, not stainable by eosin or hematoxylin, were found in them. For the present it is not possible

to identify these organic remains; I suppose that the membranes belong to connective tissue and that the yellow grains are some excretion product of the Mollusc.

13. Another accessory pearl from the same specimen (*b* in fig. 6) was dissolved. Dimensions 0.46×0.85 mm. It was very opaque and showed a granular or mosaic structure with sinuate and crenate boundary lines. The centre was occupied by a mass of calcareous granules of 0.015 - 0.017 mm diameter; a radial structure as found in spherulites was lacking. The organic matrix was dense and granular and stained by eosin.

14. Another accessory pearl from the same specimen (*a* in fig. 6) was dissolved; diameter about 1 mm. The pearl was opaque and remained so during solution. Its structure was the same as that of No. 13. The organic matrix was very dense as in No. 12 and 13, in consequence of which solution in acids occurred very slowly; it was stainable by eosin. After half of the concretion had been dissolved, the remaining mass had taken the shape of a trapezium; at first it was nearly spherical, somewhat flattened. The calcareous substance in the centre had a granular or lumpy structure (fig. 14).

The pearl was wholly covered by an organic membrane which was stained by eosin. It contained some tendinous tissue, several circumvallated pores as mentioned under No. 5, 8 and 10, and remains of foreign organisms (centric and pennate Diatoms; 3 different kinds of siliceous spicules). Many yellowish brown grains were left after solution of the calcareous matter.

15. An almost spherical concretion of 2 mm diameter, taken from the adductor-muscle, was dissolved in dilute hydrochloric acid. As the solution was in progress, the shape became more cubic. After the diameter had diminished to 0.5 mm, a distinct radial fibrous structure became visible. During solution many yellowish brown granules, not stainable by eosin, were disclosed. The organic matrix was tough and structureless. It was stained by eosin and contained a mass of the above-mentioned brown granules in its centre. Muscular or tendinous tissue was not found.

16. A concretion of 8 mm diameter with 3 places of attachment, found in a cavity of the shell near the hinge. The places of attachment are brown and contain a soft substance, which is almost wholly composed of organic matter. This organic matter (stainable by eosin) was deposited as a pile of membranes; in other places it showed a coarse granular or mosaic structure (like epithelium).

The pearl was broken. Three thin scales, split off from the surface of fracture, were dissolved in acid. One had a fibrous chevron-structure and left an organic matrix as is found in the shell, not stainable by eosin. The two others showed a granular structure, which left a dense organic matrix stainable by eosin.

When the pearl was broken into halves, the surface of fracture was like that of a piece of glue. One half was ground from beginning to end, starting from the surface of fracture. The ground surface soon showed a system of concentric growth-layers, which were developed only on one side; the remaining part was fine granular without definite structure. A wide fissure was found in the centre of the section, which in grinding on was replaced by a semi-transparent nucleus of a radial fibrous structure without growth-layers (fig. 15). The nucleus soon lost its regular shape and showed a strand of similar substance running to the surface (fig. 16). In the next section (fig. 17) only this strand of semi-transparent matter is left, which divides the section into two parts. One of these parts is provided with concentric growth-layers and a radial structure, while the other is indistinctly mottled. The strand of semi-transparent matter was partly prismatic with a fine cross-striation (0.006 mm) and connected on one side with a hollow place of attachment (fig. 17 *B*).

The second part of the broken pearl was ground into a disc, and then dissolved in acid. It showed an irregular semi-transparent nucleus and a radial structure with some faint concentric growth-layers (fig. 18). After solution was completed this nucleus left two dense pieces of organic matter (fig. 19, 20), which judging from their shape had probably filled up a cavity. The piece of fig. 20 showed a columnar structure along the rounded side and a stratification parallel to the surface.

It seems that in this concretion a cavity has been filled up by a semi-transparent calcareous substance and repair-conchyolin, and that the hollow place of attachment in fig. 17 (*B*) was connected with this cavity.

C. *Tridacna*-pearls of regular shape with white veins. These are generally sold as coconut-pearls and beautiful specimens are rather expensive. It seems that the collection bought by Dr WEBER contained only regular, bipolar, veined pearls (see p. 215). A few specimens of this kind were present among the Boeton-material, viz. two semi-globular pearls, an ovoid one with beautiful veins and a semi-transparent pearl with a cat's-eye lustre. One

of these semi-globular pearls was described and figured by BOLMAN (see p. 216). The ovoid pearl was also given to him, but not described in his monograph.

The two remaining specimens, a semi-globular pearl and one with cat's-eye lustre, were examined by Dr BOON by means of X-rays. A rotation-diagram showed a certain orientation, especially in the pearl with cat's-eye lustre, but it was not possible to point out definite zones, to measure their distance and to name the axis of orientation, which has certainly high indices. It is not likely that concentric scales of aragonite are present, as the powder-lines are incomplete. A shell-piece (nail-like projection) of *Tridacna squamosa* showed almost complete powder-lines; zones were very indistinct or absent. It should be borne in mind that in the *Pteria*-pearls of commerce the elements of prisms and nacre are symmetrically arranged around one centre, while in *Tridacna*-shells and -pearls the fibres of adjacent lamellae cross each other. Regular *Tridacna*-pearls, and probably all pearls from shells with crossed lamellae, show an axis of symmetry and are bipolar (cf. KESSEL 1937). Their symmetry is different from that of *Pteria*-pearls, which show a point of symmetry in the centre of the pearl. There is certainly a definite orientation in the crystal-fibres of the crossed lamellae of the *Tridacna*-shell, though it is not shown in the Röntgen-diagram mentioned above.

17. I have seen several regular *Tridacna*-pearls, which had been sold or presented as coconut-pearls, but only one specimen in the collection of the Indian Institute at Amsterdam (H.M. 2202-1932) was available for a detailed examination. It was pear-shaped, about 15×10 mm, rather opaque, and near the pointed end marked by white veins with a slight spiral course. Around the pointed end there were 6 small pits; the same pits were also seen in a large "mustika" of the same Institute (V.K. 189/1; see p. 217) around the place of attachment, further in shells of *Tridacna gigas* along the mantle-line.

The pearl was cut length-wise. It showed concentric growth-layers in the blunt end and two additional systems of growth-layers in the pointed end, produced by unilateral growth of the pearl. The growth-layers were crossed by white streaks radiating from the centre; they showed a feathery appearance near the circumference.

One half of the pearl was cut across to make a thin section. The calcareous matter was aragonite. The section contains about

20 nuclei of crystallization, each showing a separate spherulite-cross under the microscope in parallel polarized light. The nuclei are surrounded by dark growth-layers, similar to those found in the *Tridacna*-shell; they consist of small granules, which are dark brown or brown greenish in transmitted and white in incident light. As the nuclei are crowded in the centre, the surfaces of the growth-layers are shaped like blunt cones.

Near the circumference the section shows a strong cleavage in consequence of which the edge has partly crumbled away; the same was reported by BACON (1861) for another coconut-pearl. This part consists of layers, which alternately show their fibres lying in the plane of section and cut across, as is clearly seen under the polarization microscope. It is evident that the white veins and spots on the surface are caused by these crossing fibres; the same applies to the irregular pearl No. 9 (fig. 12). The calcareous matter was fibrous and the organic matrix jelly-like as in the *Tridacna*-shell. It seems that this pearl with its many crystallization-centres is one of abnormal structure.

18. Some powder from the large pearl mentioned on p. 217 could be examined. It was bored from a cavity in the place of attachment; the object itself could not be sacrificed, as it was a museum-specimen (Indian Institute Amsterdam, V.K. 189/1).

The outer parts of the pearl consist of concentric layers as is visible in the cavity. The calcareous matter is aragonite (fig. 28); it is rather soft and contains a large proportion of organic matter. Some spherulites were found in the cavity; in some of them the crystal fibres had been united to prisms (fig. 21). The structure of the calcareous substance was mainly prismatic. The organic matrix had a granular appearance and seemed to contain fragments of cell-tissue (epithelium and cross-stripped muscle-fibres). On closer examination, however, it appeared that these cells were the dense matrix of minute prisms, which often showed a cross-striation.

D. *Hippopus*-pearls. Only 4 small *Hippopus*-concretions were found by Dr VAN DEN BERG; they were collected from 3 different *Hippopus*-shells, which are called "rawoe rawoe" in Boeton.

One of these concretions has been described and figured by BOLMAN (1941, p. 136; pl. 34 fig. 5 a, b and c). Dimensions 3.4×1.9 mm; weight 18.4 mg; specific gravity 2.8761. Calcareous matter, according to chemical reaction (and specific gravity; R.), aragonite. The pearl showed a radial prismatic

structure (B.'s fig. 5a). The centre was occupied by a homogenous mass with fine radial fibres (B.'s fig. 5b) and surrounded by a layer of organic matter. B. interprets this nucleus as a quartz-grain, but gives no evidence that the substance is really quartz; it is similar to the calcareous nucleus of No. 16 (fig. 15). Concentric growth-layers were only visible in one place adjacent to the nucleus.

The 3 remaining specimens were only examined *in toto*. The first one, found in the adductor-muscle, is half spherical and very warty; its diameter is 3 mm. The structure is probably prismatic. Some organic matter, which was stained strongly by eosin, was found on the flat side. It was mainly amorphous, but contained some fibrous parts, which were probably muscular tissue, though cross-striation was very vague or absent.

The second specimen was also taken from the adductor-muscle. It is bluish and semi-transparent; shape almost spherical, diameter 2 mm. The structure is clearly prismatic.

A third specimen was found outside of the muscles. It is ellipsoid, 4 mm long, and provided with small warts; the colour is yellowish, opaque.

19. An abnormal tooth-like projection (fig. 22), found on the interior side of a *Hippopus*-shell and probably analogous to the free concretions described above, was examined in detail. Dimensions about $15 \times 12 \times 8$ mm; white porcellanous, somewhat transparent and provided with the same shallow grooves as found in many *Tridacna*-concretions.

The tooth contains a cylindrical cavity which extends to the tip. The surface of this cavity is brown and provided with small mamillae. The base of the tooth after grinding and etching showed concentric growth-layers and radial streaks.

The cavity was clad by spherulites (fig. 23, 24), whose structure became clearly visible after corrosion by acetic acid. Most spherulites had a diameter of 0.03–0.04 mm, the largest were 0.17 mm. Many of them were composite and assembled into aggregates. Several spherulites had tail-like projections as the fibres were partly prolonged and united to prisms (fig. 26). Many fusiform spherulites (mostly 0.035×0.070 mm) had arisen in this way; others were provided with forked tails (fig. 27). In some cases the projections had taken the shape of lamellae (fig. 25). A transformation from spherulitic to prismatic structure was very conspicuous in this object. Such transformations were also observed in some *Tridacna*-concretions.

The substance broken from the base of the *Hippopus*-tooth showed a mosaic-structure with bundles of short fibres near the cavity. A rudimentary lamellar structure with fibre-crossing was found in the outer parts.

Abnormal excrescences in *Tridacna*-shells are reported by RUMPHIUS (1705) and VAILLANT (1865).

4. Summary and discussion

The shell of *Tridacna squamosa* and *Hippopus hippopus* is built up of a single layer of crossed lamellae, which are placed perpendicularly to the secreting surface and parallel to the growth-lines. The width of the lamellae ranges from 0.03–0.04 mm. Their edges are visible on the inner surface of the shell as parallel dark and white veins, which are partly connected with each other and run parallel to the distal edge (and growth-lines) of the shell. In radial sections the edges of the lamellae are seen as white and dark veins, which are slightly forked and in the upper parts bent umbo-ward (fig. 4). Parallel to the secreting surface (and perpendicular to the lamellae) there is a system of dark growth-layers composed of minute granules, which are white in incident and dark brown or brown greenish in transmitted light. In tangential sections the crossing fibres meet these growth-layers at an angle of about 45° (fig. 3). The outer surface of a young shell of *Tridacna squamosa* is covered by a thin periostracum which is strongly stained by eosin. According to Röntgen-diagrams this shell consists of aragonite; its specific gravity is about 2.77.

A hypostracum on the muscle-impressions was not found; the veins on the inner surface of the shell pass without any interruption over the impressions.

A distinct prismatic structure is present in the conchyolin-layer next to the ligament; the prisms show a fine cross-striation. Some parts of the hinge have a more or less distinct prismatic structure.

The shells contain only a small proportion of organic matter, probably less than 3 per cent, left as a jelly-like mass after solution of the calcareous substance. The granules of the growth-layers contain a pigment which is solved in acids; the solution of CaCO_3 is slowed down by their presence.

The structure of the organic and calcareous matter in *Tridacna*- and *Hippopus*-pearls reveals little about their origin. As the process of normal and abnormal shell-secretion (repair of lesions)

has never been studied in *Tridacna*, it is difficult to understand the production of abnormal shell-secretions like pearls. It is, however, quite certain that the majority of the *Tridacna*-concretions is found in the muscles, especially near the edge of their insertions (RUMPHIUS 1705; observations of Dr E. J. VAN DEN BERG).

Muscle-pearls in nacreous shells have been examined by RUBBEL (1912) in *Margaritana* and by JAMESON (1912) in *Pteria*, but their origin is yet imperfectly known. They consist of hypostracum, a modification of nacre found on the muscle-insertions. These muscle-pearls are very small (0.02-0.5 mm) and often crowded in large numbers; RUBBEL found over 400 in one single section of 2×2.5 mm in *Margaritana margaritifera* (Linn.). According to JAMESON the muscle-pearls of *Pteria vulgaris* (Schum.) arise where the muscle-attachment epithelium passes over into the nacre-secreting mantle-epithelium. They are formed around a central cavity, which is lined by hypostracum, ordinary nacre, or repair-substances analogous to those which occur where the normal shell-secreting processes are disturbed. In the muscle-pearls of *Margaritana* the centre is occupied by yellow grains; cavities seem to be absent (RUBBEL). JAMESON states that the hypostracum-pearls are often overgrown by nacreous layers and connected by a hilum of hypostracum to the muscle-attachment epithelium; their irregular shape is largely due to crowding and the presence of these hila. JAMESON is of opinion that the muscle-pearls arise by some pathological invagination or immigration of the epithelium in the region where the muscle-attachment epithelium passes into mantle-epithelium, and that this may be due to the action of parasites, since muscle-pearls occur only locally. The histological changes in the above-mentioned region during displacement of the muscles in the growing shell have never been studied. A pearl-sac is often imperfectly developed or absent in muscle-pearls; in many cases they lie loosely in connective tissue.

Common mantle-pearls in nacreous shells (*Unio*, *Anodonta*, *Pteria*) can be artificially produced by injection of pieces of mantle-epithelium into the connective tissue of the mantle (ALVERDES 1913). In cases where foreign bodies like sandgrains and parasites are found in the nucleus of pearls, it is likely that these bodies have caused pearl-formation by carrying mantle-epithelium into the interior of the mantle. As a rule the cause of pearl-formation in nature is unknown; foreign nuclei are rather

exceptional. It seems that pearl-formation is commonly associated with some lesion of the epithelium in which an epithelial luxuriation forms the pearl-sac (cf. ZAWARZIN 1927). Pearls have even been found in the wall of the visceral mass and in the ovarium (JAMESON 1912). In shells with crossed lamellae the development of pearls has never been studied.

At present it is generally accepted that the shell is produced by a fluid secretion of the mantle, as was suggested long ago by RÉAUMUR (1717) (JAMESON 1912, BIEDERMANN 1914). The lime-salts crystallize in a colloidal albuminous medium; both are secreted by the mantle. HARTING (1872) and others have obtained structures similar to those of Mollusc-shells by having lime-salts crystallized in egg-albumen. A new theory of shell-formation, at variance with the preceding one, was published by DE WAELE (1927).

Tridacna-pearls agree with muscle-pearls of *Pteria* in so far, that they are commonly found in the muscles and that they are often of irregular shape and clustered in aggregates. A pseudo-nucleus, similar to the hypostracum-pearls described by JAMESON and RUBBEL, has not been found in *Tridacna*. The centre of *Tridacna*-pearls is usually occupied by a more or less amorphous calcareous mass, which is often opaque, but in some cases semitransparent and provided with a faint radial striation. The surrounding parts show mostly concentric growth-layers in the larger specimens; in very small pearls (1 mm and less) they are absent.

Organic membranes, staining with eosin, are often found on the surface of *Tridacna*-pearls, which have been collected from the muscles. These membranes are almost structureless; only in a few cases muscular and tendinous tissue was observed in them.

The organic matrix of *Tridacna*-concretions often varies in density in different parts of the pearl. Dense organic matter is generally associated with a prismatic structure; on free surfaces spherulites are developed. A dense organic matrix often shows the structure of the calcareous mass after the CaCO_3 has been dissolved. When the calcareous matter is fibrous a jelly-like matrix is left, just as in the shell; it does not stain with eosin as the dense organic matrix generally does. A large proportion of organic matter, probably repair-conchyolin, is found on the places of attachment, which are common in *Tridacna*-concretions and sometimes provided with a cavity.

The calcareous matter shows many intermediate forms between

spherulites and crossed lamellae, similar to those in the repair-substance of shell-lesions in Gastropods (BIEDERMANN 1914, 1917; ANDREWS 1935). The irregular *Tridacna*-concretions are largely built up of repair-substance. Only in the outer parts of regular pearls the crossed lamellar structure of the shell has often clearly developed as is shown by white veins on their surface. These veins are the edges of crossed lamellae, which are placed vertically to the secreting epithelium and surface of the pearl. In irregular pearls the venation has seldom developed; regular pearls are usually veined or mottled due to crossing fibres.

Foreign nuclei have not been found in *Tridacna*-pearls. In a few cases remains of foreign organisms (eggs, cysts, Diatoms, siliceous spicules) were incorporated in the calcareous mass, but probably only by accident. Some of these remains were also found on the mantle of *Tridacna squamosa*, but not in the shell. Sometimes cavities are found in the interior of *Tridacna*-pearls; they are lined by organic membranes, which are often covered by spherulites.

With reference to the studies of JAMESON (1912) and RUBBEL (1912) on muscle-pearls and my own observations on *Tridacna*-concretions I conceive the formation of muscle-pearls in *Tridacna* for the present as follows. The muscle-attachment epithelium near the edge of the muscles, which shift during growth of the shell, is detached for some cause in an irregular way, so that small cavities are formed, which are overgrown by the intruding mantle-epithelium (cf. ZAWARZIN 1927). These cavities, which have usually an irregular and more rarely a regular shape, are filled up with organic and calcareous matter produced by the intruding mantle-epithelium. Sometimes a part of the cavity is left (No. 7, 8, 9, 16, 19). In some cases the connection with the shell is permanent so that an excrescence is formed (No. 18, 19). The concretions are usually severed from the shell, but show often traces of former attachment (45 % of the 42 concretions mentioned on p. 218). As the pearl grows the shape becomes more and more rounded, while concentric growth-layers have developed. Sometimes adjacent pearls are united in consequence of which aggregates are formed (No. 4, 5, 10; p. 214 and 218). The final result is a structure similar to the crossed lamellae of the shell (No. 9, 17), characterized by white veins on the surface. As the surface is strongly curved, the pearl has a tendency to cleave, especially in the outermost parts; cracks are common in *Tridacna*-

concretions (in 40 % of the 42 concretions mentioned on p. 218). The nature of the crossed lamellae involves an arrangement of the crystal-fibres around an axis of symmetry, so that regular *Tridacna*-pearls are bipolar. In pearls from shells composed of nacre and prisms, the crystal-fibres are arranged around a point of symmetry, the centre of the pearl. The two poles of a *Tridacna*-pearl are often different in shape and structure of the calcareous substance (venation, transparency). The shallow, parallel grooves, found in 55 % of the 42 concretions mentioned on p. 218, are possibly caused by the presence of muscle-tendons; the edge of a *Tridacna*-adductor is wholly tendinous.

The above-mentioned conception is merely a hypothesis, but it explains some peculiarities of *Tridacna*-pearls, their irregular and aberrant forms (e.g., semi-globular pearls), the prevalence of places of attachment, their irregular structure largely of repair-substance and possibly the common occurrence of shallow, parallel grooves. A study on the spot, combined with an examination of sections of the muscle-edge would be needed to throw more light on the origin and development of *Tridacna*-pearls. The muscle-attachment epithelium, the calcareous layers produced by it, and its change into common mantle-epithelium still wait investigation. Our knowledge of muscle-pearls in general is still very incomplete, especially with regard to their origin.

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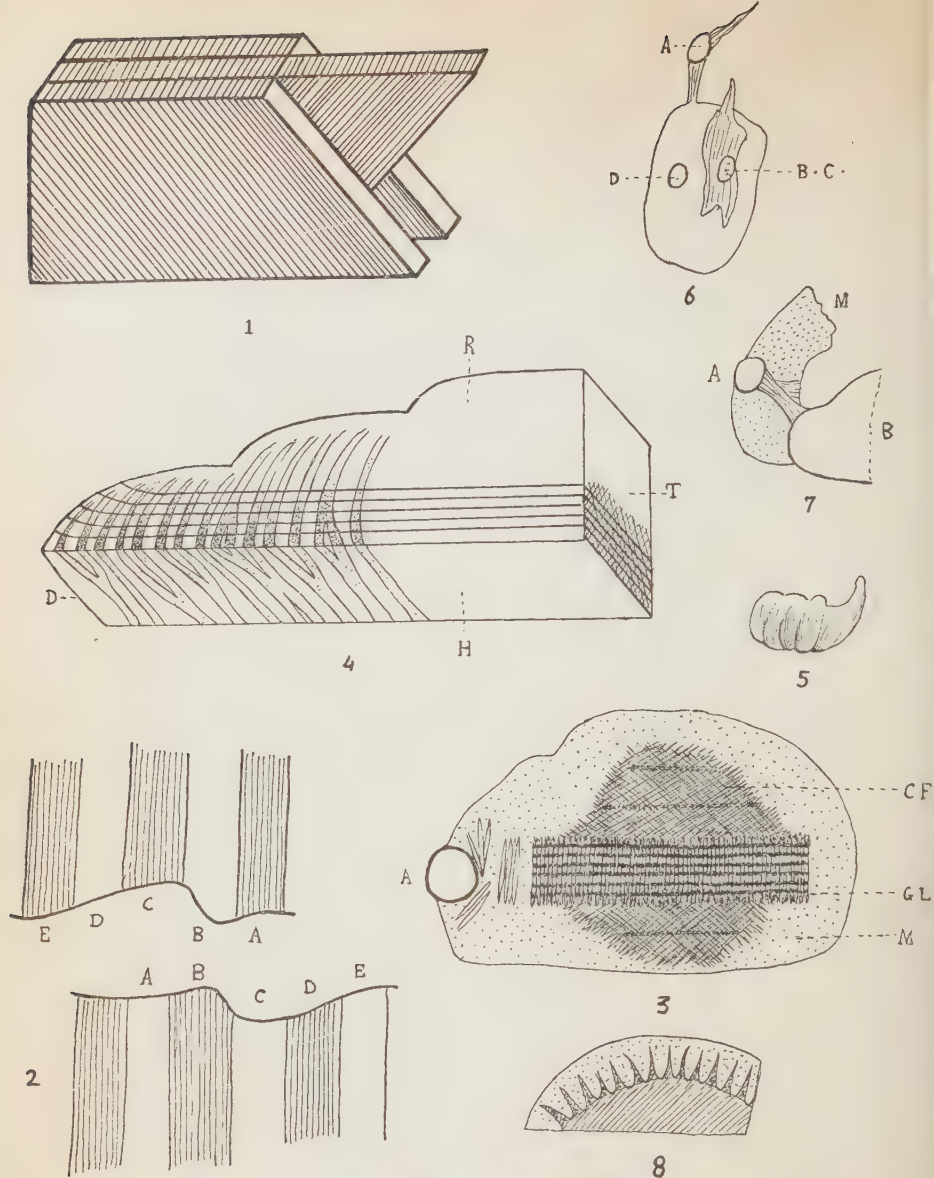


Fig. 1. Crossed lamellar structure; 3 lamellae I, composed of lamellae II which cross each other at an angle of about 90° (copied from EØGGILD 1930). Fig. 2. Part of a radial section of the shell of *Tridacna squamosa*; A-E white and dark veins (edges of lamellae I). After turning the section 180° the white veins become dark and the dark veins white ($200\times$). Fig. 3. Tangential section of the shell of *Tridacna squamosa*, corroded with acetic acid; CF crossed fibres, GL dark growth layers with fine prismatic structure; M jelly-like organic matrix; A CO_2 -bubble (schematized). Fig. 4. Diagram of the shell-structure of *Tridacna squamosa*. R Radial section; D distal edge of the shell; H Horizontal section (inner side of shell); T tangential section. In R and T some horizontal growth-layers are drawn. Fig. 5. An irregular concretion of *Tridacna squamosa* with a crest-like excrecence ("retort"-shape of RUMPHIUS). Fig. 6. A concretion from the adductor-muscle of *Tridacna squamosa* with 3 accessory pearls (A, B, C), attached to the mother-pearl by membranes, and 2 pearls imbedded in its mass (D; the second, on the back side, is not visible in the figure). Fig. 7. Accessory pearl (A) fastened by a membrane (M) and a membranous strand to the mother-pearl (B). Fig. 8. Irregular concretion of *Tridacna squamosa*, partly dissolved in acid. In the jelly-like organic matrix (dotted in the figure) spines of denser organic substance are visible.

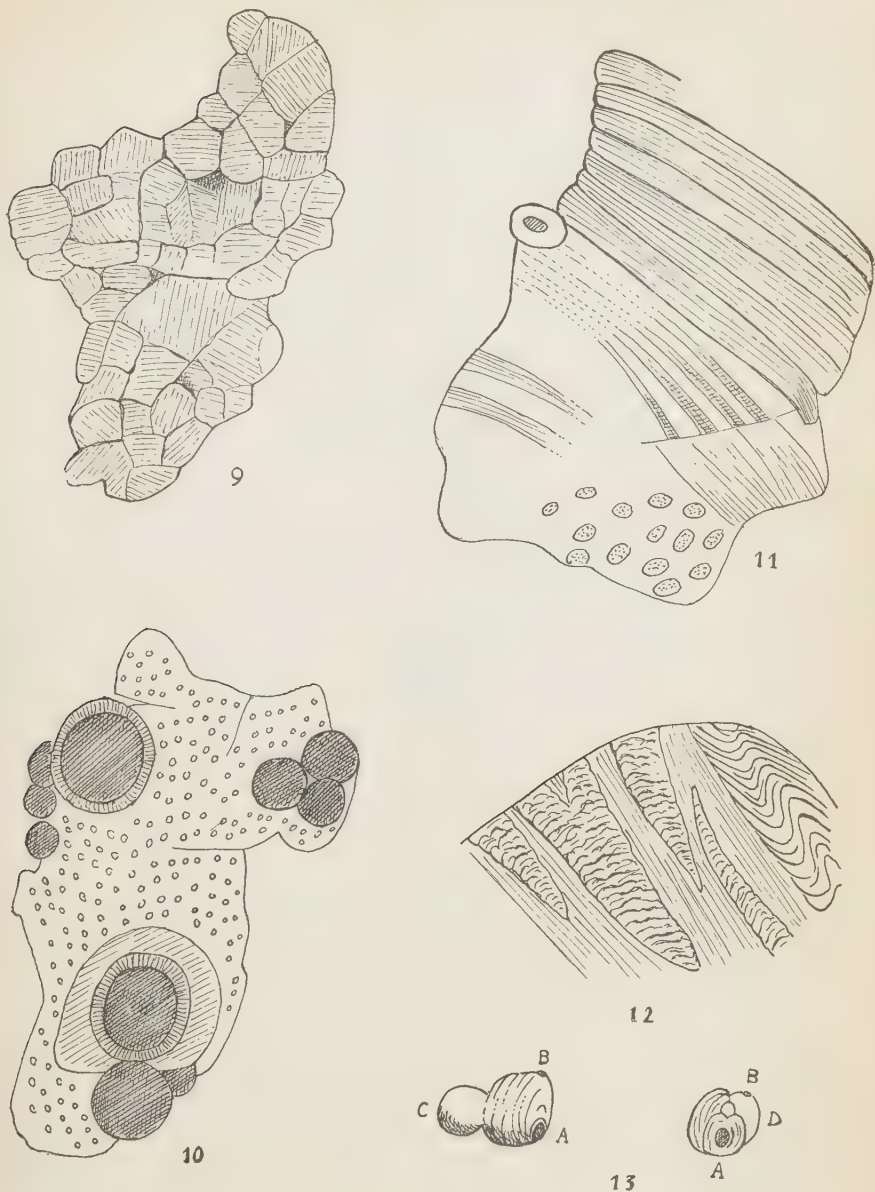


Fig. 9. Mosaic-structure in pearl No. 6 ($200\times$). Fig. 10. Organic substance lining the cavity in pearl No. 8 (after solution of the calcareous matter). Remains of spherulites on a brittle membrane with eosinophilous granules ($97\times$). Fig. 11. Columnar structure in the dense organic matrix of pearl No. 8. On the left side a circumvallated pore. Below: eosinophilous granules. ($450\times$). Fig. 12. Coarse crossed lamellae in pearl No. 9; on the right side a chevron structure ($97\times$). Fig. 13. Concretion No. 10 with 2 glazy pearls *C* and *D* and 2 places of attachment *A* and *B*.

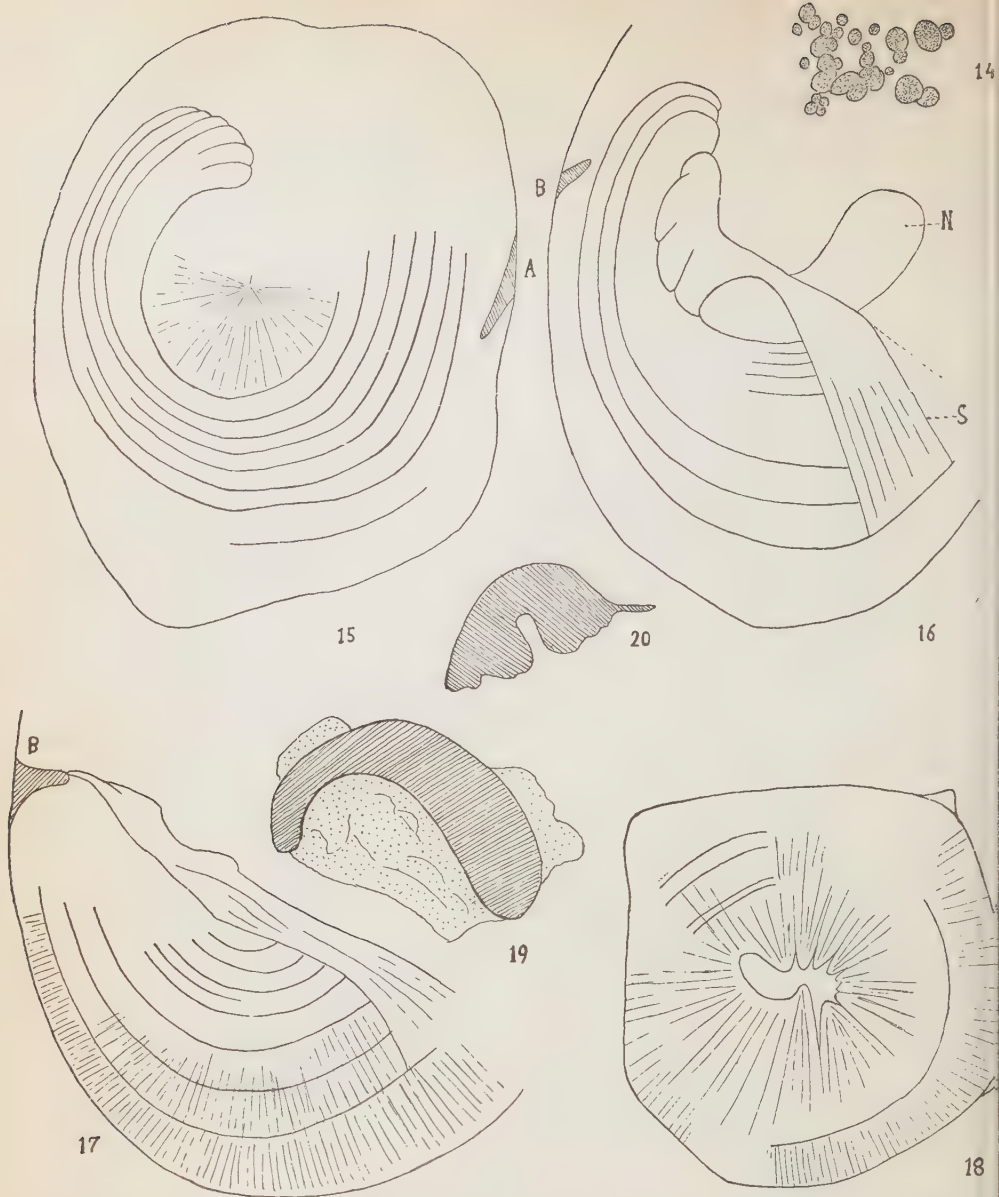


Fig. 14. Centre of an accessory pearl (*A* in fig. 6) occupied by amorphous granules of calcareous substance ($150\times$). Fig. 15-17. Three successive sections through one half of pearl No. 16, starting at the centre (fig. 15). Fig. 15. Pearl No. 16. Section (near the centre) with a transparent nucleus. The growth layers and nucleus have only developed on one side; *A* a place of attachment ($12\times$). Fig. 16. A section of pearl No. 16, following the preceding one; *N* transparent nucleus; *S* strand of transparent matter, not connected with the place of attachment (*B*) ($12\times$). Fig. 17. A section of pearl No. 16; following the preceding one. The growth layers have only developed on one side. A broad strand of transparent calcareous matter is connected with the place of attachment (*B*) ($12\times$). Fig. 18. A section of the second half of pearl No. 16, corroded by acid. A transparent nucleus of irregular shape and a radial structure; with faint growth-layers near the circumference are visible ($12\times$). Fig. 19 and 20. Pearl No. 16. Pieces of dense organic matter which were probably deposited in a cavity ($32\times$). In fig. 20 there was a columnar structure on the rounded side and a stratification parallel to the surface. These pieces of organic substance were left after solution of the calcareous matter in the section of fig. 18.

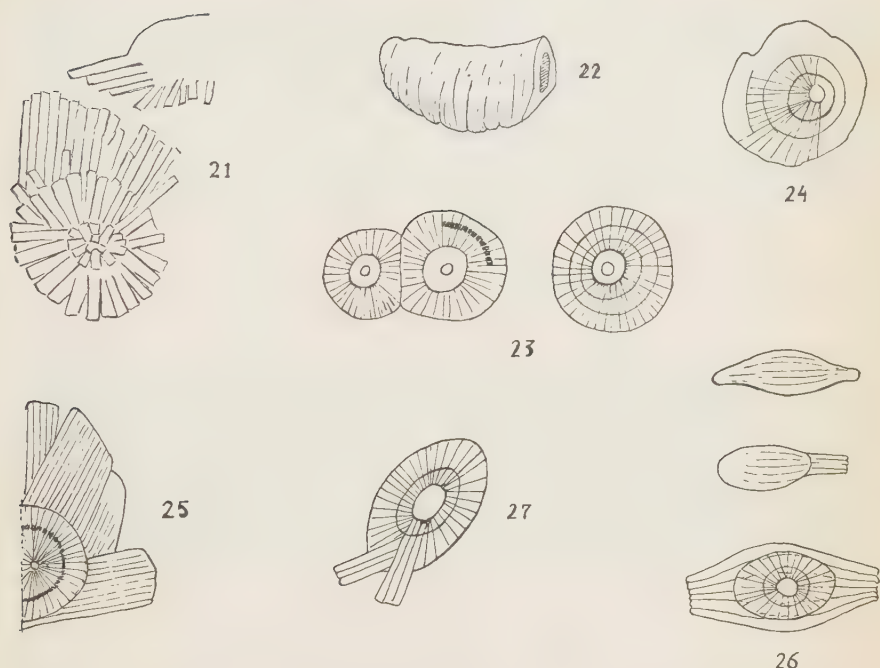


Fig. 21. A spherulite, in which the fibres are united to prisms; from the cavity of No. 18. ($330\times$). Fig. 22. A hollow tooth-like excrescence on the inner side of the shell of *Hippopus* (No. 19). Fig. 23 and 24. Spherulites lining the cavity of No. 19; in fig. 24 the fibres have only developed on one side. Fig. 25. A spherulite from the *Hippopus*-excrecence (No. 19) with the fibres partly developed into lamellae. Fig. 26. Spherulites from the same object, partly transformed into a prismatic structure, and spindle-shaped. Fig. 27. A spherulite from the same object with a forked-tail.



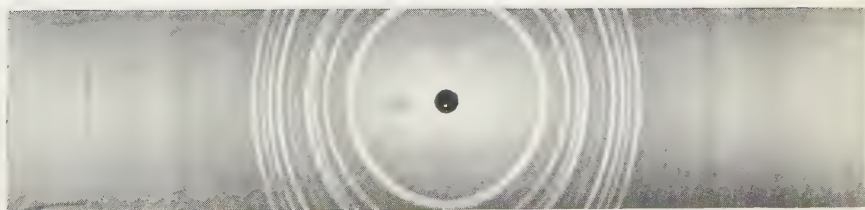
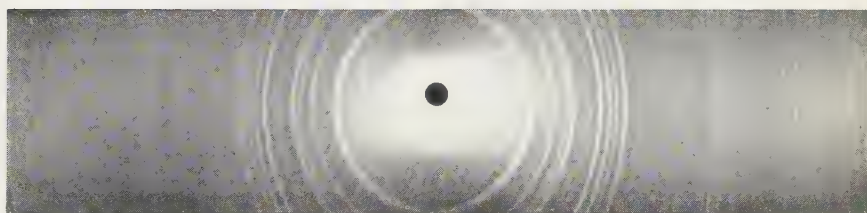
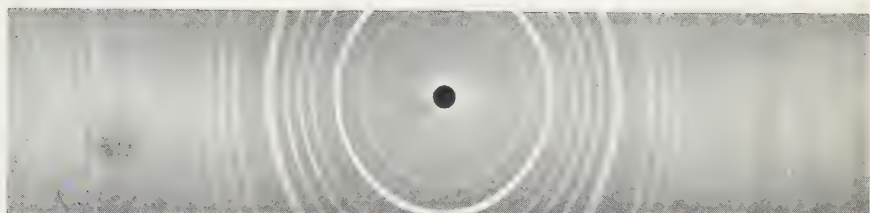


Fig. 28. *Debye-Scherrer* diagram of calcite (upper figure), aragonite and pearl No. 18 (lower figure). Photographs by Dr J. W. Boon.

OBSERVATIONS ON A CALVARIUM OF *EQUUS*
SIVALENSIS FALCONER ET CAUTLEY
FROM THE SIWALIKS OF THE PUNJAB, WITH
CRANIOMETRICAL NOTES ON RECENT
EQUIDAE

BY

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with pl. IX and two textfigures

In March 1895, after having made an extensive collection of fossil Vertebrates in Java, the description of which, after long delay, is now proceeding, EUG. DUBOIS made a trip to India, and collected Vertebrate fossils in the vicinity of Haripoor on the Somb Nuddy, Sirmur State, in the Punjab. The Siwalik collection of DUBOIS contains skulls of *Hippopotamus sivalensis* Falconer et Cautley, of *Hippopotamus* nov. spec. (the description of which is forthcoming), a lower jaw of *Aceratherium perimense* Falconer et Cautley, and still undetermined material of *Elephas*, *Stegodon*, and ruminants. Remains of tortoises also are with the collection.

The object of the present paper forms the calvarium of a horse, which was found at Jhil, ca. 2 miles E.S.E. of Haripoor.

Fossil remains of *Equus* from the Siwaliks and the Narbada beds were figured in the Fauna Antiqua Sivalensis by FALCONER and CAUTLEY (1849, pls. 81-85) under three different names, viz., *Equus sivalensis*, *E. namadicus*, and *E. palaeonus*. In the Palaeontological Memoirs of FALCONER (1868, pp. 524-531) we find the measurements of the figured specimens (which for the greater part are in the British Museum), and also (l.c., pp. 186-188) descriptions of other Siwalik specimens in the Museum of the Asiatic Society of Bengal ¹⁾. LARTET (vide l. c., p. 22, 186 foot-notes) suggested *E. palaeonus* to be only a young of *E. sivalensis* or *E. namadicus*. LYDEKKER (1882, p. 96) also is of the opinion that *E. palaeonus* is not a distinct species, and regards it as synonymous

¹⁾ Copied from FALCONER and WALKER (1860, non vidi).

with *E. namadicus*. To the latter species he also refers (l. c., p. 94) a right maxilla from the Siwaliks, figured as *E. sivalensis* in FALCONER and CAUTLEY (1849, pl. 82 fig. 1). This is apparently the same specimen as that represented, l.c., pl. 81 fig. 3, which is likewise omitted under the heading *E. sivalensis* in the British Museum catalogue (LYDEKKER, 1886, p. 67).

Equus sivalensis was traced into Mongolia by LYDEKKER (1891, p. 210/11) on the evidence of two separate fossil teeth. He does not hesitate to refer the two teeth said to be from Yunnan and described and figured by KOKEN (1885, p. 49/50, p. I figs. 14-15) to the Siwalik species too.

LYDEKKER maintained the two fossil Indian forms of *Equus* as separate species, the Narbada form having more specialized characters as compared with *E. sivalensis*. MATTHEW (1929, p. 530/31) regards their distinction as doubtful, though *E. namadicus*, as of younger geological age, may be a progressive form of *E. sivalensis*. This is also the opinion of COLBERT (1935, p. 162).

The lower tooth-series represented on pl. 82 fig. 2 of FALCONER and CAUTLEY (1849) as *E. sivalensis* was made the type of a new species, *E. cautleyi*, by HOPWOOD (1936, p. 907) as the only Siwalik member of the zebrine group¹).

The examination of the present Punjab specimen, and the study of the literature and of the variation in recent horses brought me to the conclusion, that *E. namadicus* has no right to specific distinction from *E. sivalensis*, so that the former name is merely a synonym of *E. sivalensis*.

For comparison purposes I used a series of skulls in the Leiden Museum and in the Zoological Museum at Amsterdam. Many authors have dealt with the cranial characters of the Equidae, especially the differences between horse and ass; DUERST (1908), OSBORN (1912) and PETIT (1937) give useful reviews. As my material has not been used before for a craniometrical treatise, I composed a table of measurements (table I, mainly after OSBORN (1912), with some additions), which may serve as a supplement to the tables of measurements already published by, e.g., FRANCK (1875), BRANCO (1883), NEHRING (1884), TSCHERSKI (1892), SALENSKY (1902), ANTONIUS (1913) and MOTOHASHI (1930). In few cases only the exact age of a skull was recorded. The age of

¹) The term zebrine was not first used by DARWIN in 1868 (see HOPWOOD, 1936, p. 900); it is found already in OWEN (1846, p. 385).

the other specimens was estimated by means of the data provided by veterinary works. I measured only skulls in which M^3 is already in use. The numbers given to the specimens in the list below correspond to those mentioned in the table.

Equus caballus L.

1. Skeleton of female. Leiden Museum, cat. a. Europe. Age seven years.
2. Skeleton of male. Leiden Museum, cat. b. From the Cabinet d'Anatomie 1860. Age eight years.
3. Skull of male. Leiden Museum, cat. c, don. EKEMA. Age twelve to eighteen years.
4. Skull of female. Leiden Museum, cat. d, same donor. Age five years.
5. Skull of male. Leiden Museum, cat. e. From the Cabinet d'Anatomie 1860. id_3 not yet shed, I_3 erupting; age four to five years.
6. Skull of male, eleven years old. Leiden Museum, cat. h. The „Phénix” of B. LOISET.
7. Skull of male. Leiden Museum, cat. k. Age fifteen to sixteen years.
8. Skull of female. Leiden Museum, cat. l. From the Cabinet d'Anatomie 1860. Age seven years.
9. Skull of male. Leiden Museum, reg. no. 2603, don. D.J. KIENJET 8-1-1937. Age twelve years.
10. Skull of female. Leiden Museum, reg. no. 3199, same donor, 27-9-1937. Age twelve years.
11. Skull of female. Leiden Museum, cat. m. From the Cabinet d'Anatomie 1860 (= cat. c of *E. hemionus* Pallas in JENTINK, 1887, p. 159). Age ten years.
12. Skull of female. Leiden Museum, reg. no. 4943. Pandan, Java, don. E. DUBOIS 1941. Age nine years.

The measurements of the mule (*E. asinus* ♂ x *E. caballus* ♀) no. 1 are taken from a male skeleton in the Leiden Museum, reg. no. 973, don. J. H. JURRIANSE, Rotterdam, 5-2-1920. Age ten years. Those of the second specimen I copied from NEHRING (1884, p. 100/101). It is also a male, forty years old.

The measurements of the hinny (*E. caballus* ♂ x *E. asinus* ♀) are given by SALENSKY (1902).

Equus zebra L.

1. Skeleton of female. Leiden Museum, cat. a. Cape. P^4 and M^3 have just been taken into use (this would point to an age of about five years: (OSBORN, 1912, p. 60), but id^2 and id^3 are still present. Age three to three and one-half years.
 2. Skeleton of male. Leiden Museum, cat. a (as *E. montanus* Burchell). Cape, don. VAN AKEN. Age twenty-four to thirty years.
 3. Skull of male. Amsterdam Museum, from the Amsterdam zoological garden 16-8-1903. Nasals damaged. Age nine years.
- The cranial measurements of two other zebras I copied from SALENSKY (1902).

Equus asinus L.

1. Skull of male. Leiden Museum, cat. b. Europe. Age twenty-four to thirty years.
2. Skull of female. Leiden Museum, cat. c. Europe. Don. VAN LIDTH DE JEUDE 1866. I³ sin. abnormally shaped. Age eight years.
3. Skull of female. Leiden Museum, cat. e. Djeddah, don. J. A. KRUYT 1880. Age fifteen years.
4. Skull of male. Leiden Museum, cat. f, same history. Age twenty-four to thirty years.
5. Calvarium and lower jaw, not belonging to one and the same individual. Leiden Museum, cat. g, same history. Age of calvarium about nine, of lower jaw over twelve years.
6. *Equus asinus africanus* Fitzinger. Skeleton of female, eleven years old. Leiden Museum, reg. no. 756. From an animal born in 1906, and received from the Rotterdam zoological garden 22-5-1917.
7. *Equus asinus taeniopus* Heuglin. Skeleton of male. Leiden Museum reg. no. 1441. From the Rotterdam zoological garden 10-12-1925. Age twenty-four to thirty years.

The range of variation of the measurements of three skulls of *E. asinus somaliensis* Noack, given by MOTOHASHI (1930, tables 10, 13, 15 and 16) is given in the last column of the present species.

Equus hemionus Pallas

1. Skull of male. Leiden Museum, cat. a. Tibet, from B. H. HODGSON 1853. Base of cranium missing. Age nine to ten years.
2. Calvarium of female, with a lower jaw not belonging to the same individual. Leiden Museum, cat. b. Transbaikalia, from the St. Petersburg Museum 1862. The lower jaw has well developed canines; the left I₃ is missing, and there is no trace of its alveolus. Age of calvarium less than nine years, of lower jaw ten years or more.
- N.B. The skull from the Cabinet d'Anatomie received in 1860 and listed under the present species as cat. c by JENTINK (1887, p. 159) is *Equus caballus* L.
3. Skeleton of male. Amsterdam Museum, no. 509. Teeth irregularly worn. Age probably over twenty years.
4. Skull of male. Amsterdam Museum, no. 524. Age five years.
5. Skeleton of male, over twenty-one years old. Amsterdam Museum, from an animal that lived in the Amsterdam zoological garden from 1916 to 22-9-1937.
6. Skull of female, twenty-one years old. Amsterdam Museum, from an animal born in the London zoological garden in 1918, received at Amsterdam in 1922, and died in the Amsterdam zoological garden 27-8-1939.
7. The measurements of a Pleistocene skull from Sjara-osso-gol, Ordos, N. China, referred to the present species by BOULE and TEILHARD (1928, p. 39). Finally I added the range of variation of nine adult skulls measured by SALENSKY (1902), and that of twelve adult specimens of which MOTOHASHI (1930, tables, 8, 11, 15 and 16) gives the measurements.

Equus ferus Pallas (= *E. przewalskii* Polj.¹)

1. Skeleton of male. Leiden Museum, reg. no. 359. Imported by HAGENBECK, don. F. E. BLAAUW 10-9-1914. Age twelve years.
2. Skeleton of female. Leiden Museum, reg. no. 1534. Same history, received 23-9-1926. Age eighteen to twenty-four years.
3. Skeleton of male. Amsterdam Museum, from the Amsterdam zoological garden 9-2-1938. Age twelve to eighteen years.
4. Skull of female, twelve years old. Amsterdam Museum. „A yellow dun mare out of a dun Mongolian mare by a Przewalski stallion”, presented by Prof. J. C. EWART 14-12-1920, then eight years old. Died in the Amsterdam zoological garden 22-8-1924.

Both SALENSKY (1902) and MOTOHASHI (1930, tables 9, 12, 15, and 16) give measurements of five adult specimens, the range of variation is given in the present table.

Equus grevyi Oustalet

1. Skeleton of female. Leiden Museum, reg. no. 885, don. F. E. BLAAUW 23-10-1918. Age eighteen years.
2. Skull of male. Amsterdam Museum, from the Amsterdam zoological garden. Age six years.
3. Skull of female. Amsterdam Museum, from the Amsterdam zoological garden. The right M³ is rotated, with its outer surface facing forward. id₃ not yet shed; age four to five years.

The measurements of a fourth skull of the present species I copied from ANTONIUS (1913).

Equus quagga Gmelin*Equus quagga quagga* Gmelin

1. Skeleton of male. Leiden Museum, cat. a. Cape, from M. VAN HORSTOCK 15-6-1827. Age six years.
2. Skull of female, over sixteen years old. Amsterdam Museum, no. 522. From an animal that lived in the Amsterdam zoological garden from 9-5-1867 to 12-8-1883. Mounted skin figured in Proc. Zool. Soc. London, 1904 I, p. 430 fig. 86.

Equus quagga burchellii Gray

3. Skeleton of female. Leiden Museum, reg. no. 1513. From the Rotterdam zoological garden 4-8-1926. Age twelve (?) years.
4. Skull of male. Amsterdam Museum, no. 529. Age seven years.
5. Skull of female. Amsterdam Museum. Age ten (?) years.

Equus quagga chapmani Layard

6. Skeleton of female. Leiden Museum, cat. a. From the Rotterdam zoological garden 20-8-1907. There is no trace of the left I³ or of its alveolus. The left M₁ is displaced inwards. Age twelve years or more.

¹) See ANTONIUS, 1913, p. 271.

7. Skeleton of male, twenty years old. Leiden Museum, reg. no. 2284. From the Rotterdam zoological garden 16-11-1934. From an animal born in the Amsterdam zoological garden in 1914. The left upper C is missing.
8. Skull of female, more than ten years old. Amsterdam Museum, from the Amsterdam zoological garden 3-2-1940. Purchased from L. RUHE, Alfeld, Germany, 26-6-1930. Between the upper central incisors there is a small malformed extra incisor (see HOOIJER, 1945, p. 103, fig. 1).

Equus quagga granti De Winton.

9. Skeleton of male. Leiden Museum, reg. no. 2204. From the Rotterdam zoological garden 7-12-1933. Showing "shear-bite", like in the skull figured by COLYER (1936, p. 630-633, figs. 852-854), on the left side. The wear of the incisors is normal. Age ten years.
10. Skull of male. Coll. CARP (Amsterdam Museum), from an animal shot at Margereni, in March 1939. Age four to five years.

Finally I give the range of variation of four adult skulls of the latter subspecies, measured by MOTOHASHI (1930, tables 9, 12, 15 and 16).

Reverting now to the table of measurements, it will be seen that there is a great variation in size within the species. The largest skull of *E. caballus* measured by me (no. 10) has a basilar length of 574 mm, and also has the greatest vertex length (625 mm). The mule (no. 1) stands second for the vertex length (618 mm), but its basilar length (536 mm) is exceeded by not less than seven horse-skulls (nos. 3, 4, 7-10). A skull of *E. grevyi* measured by ANTONIUS (1913), in which the vertex length (630 mm) is greater than in the largest horse-skull of my collection, is equalled in the basilar length by the second largest horse-skull (no. 2). The differences between the vertex and basilar length, alluded to by NEHRING (1884, p. 103), depend on the greater or lesser prominence of the occipital crest, a good character (OSBORN, 1912, p. 90) to distinguish between horses, asses and zebras, to which I shall again refer when discussing the occiput-vertex angle.

The largest skull of the domestic horse recorded in literature is a female Pinzgauer (ANTONIUS, 1913, p. 300, age not given) with a basilar length of 640 mm; the smallest adult a fifteen years old Exmoor pony (NEHRING, 1884, p. 98) with a basilar length of 390 mm. The mule in the Leiden Museum and that of NEHRING's table fall within this range of variation, and are larger than *E. asinus*, even than the Somali wild asses measured by MOTOHASHI, which present greater dimensions than the skulls of the domestic ass measured by me. The hinny of SALENSKY's table is not larger than the ass, but exceeds the small Javan horse of my collection (no. 12) in size.

E. zebra is very variable in size, the variation in the five skulls of my table is even greater than in the in total twenty-eight specimens of *E. hemionus*. Though SALENSKY and MOTOHASHI disposed of more skulls of the latter species than I do, among those measured by me there is one (no. 3) smaller than either of the others. This skull stands somewhat apart in other characters too, except those apparent from the table, e.g., in the high position of the infraorbital foramen (p. 258) and in the strongly developed lacrimal tubercle (p. 260). The fossil skull of *E. hemionus* (no. 7) is seen to fall completely within the range of variation of the recent specimens; the dental index (40) only is greater than in the others (32-39). The length P^2-M^3 is exceeded only by one of SALENSKY's specimens; some of the measurements of the teeth, however, are greater than those in the recent skulls (see below, p. 262).

Of the skulls of *E. ferus* measured by me, two (nos. 3 and 4) are slightly larger than the ten of SALENSKY and MOTOHASHI. Skull no. 4 is labelled as the cross between *E. ferus* ♂ and a Mongolian mare presented by Prof. EWART, but the identity of the skull is said to be not certain. I can find no characters to separate it from *E. ferus*. ZDANSKY (1935, pp. 48-51, pl. VII fig. 5) refers to *E. ferus* an incomplete and hardly fossilized calvarium from Tomenlo in Mongolia. Except for the greatest width of the cranium (which is given as 115 mm, thus slightly greater than in *E. hemionus* (93-112 mm) and in *E. ferus* (96-110 mm) as shown in my table I), and the width between the facial crests (after FRANCK and NEHRING, not characteristic) no craniometrical data are given. His identification is based on dental characters. The specimen, however, exhibits some characteristic features overlooked by ZDANSKY, which support his identification. As pointed out by MOTOHASHI (1930, p. 33 and 34, tables 8 and 9), in *E. ferus* the condylar width and the distance from the basilar tubercles to the foramen magnum are greater than in *E. hemionus*. This is shown in the table below, in which I give the range of variation in

	<i>E. ferus</i> Pallas			<i>E. hemionus</i> Pallas	
	MOTOHASHI (1930)	present author	ZDANSKY (1935)	MOTOHASHI (1930)	present author
Condylar width	74-80	77-88	ca. 81	66-76	72-77
Distance from basilar tubercles to foramen magnum	65-72	61-74	ca. 63	42-54	45-51

MOTOHASHI's material, and that found by me. The measurements of ZDANSKY's specimen as taken from the figure are seen to fall within the range of variation of *E. ferus*, and above that of *E. hemionus*.

Among the ten skulls of *E. quagga* I found no decisive characters for the different subspecies. The four skulls of *E. quagga granti* of MOTOHASHI have smaller basilar lengths than any of them.

In the following it has only been my intention to review some of the well-known and often-cited craniometrical differences between horse and ass as far as they are apparent from the table, and to see whether they are also characteristic for the other species.

A very important index is the cephalic index. In my material of *E. caballus* it varies from 39 to 44, in *E. asinus* from 45 to 49. Thus in the ass the frontal is proportionally wider than in the horse. The mule, and also the hinny (44, 42 and 41) resemble the horse. NEHRING (1884, p. 107) divides the domestic horses into two groups, the broad-headed with an index (I)
$$\frac{\text{basilar length} \times 100}{\text{frontal width}}$$

below 240, and the narrow-headed with the latter index above 240. Other divisions have been proposed, but NEHRING's dividing line, at a cephalic index of 42, appears to be very useful, as far as concerns my material. So *E. ferus* (42-45) and *E. hemionus* (42-50) are broad-headed, *E. grevyi* (36-42) narrow-headed. The quagga (39-46) and the zebra (39-48) almost overlap both the horse and the ass.

The facio-cephalic index offers no decisive character to distinguish between horse (75-78) and ass (74-80); the mule (79) agrees with the ass. Also no important dividing line can be traced for the other species, though in *E. hemionus* (79-83) the face is proportionally longer than in *E. grevyi* (76-78) and in the ass. For the zebra (76-80), *E. ferus* (76-80) and the quagga (75-81) we find almost the same values.

The cranio-cephalic index is again of importance. The cranium is proportionally longer in the ass (39-45), in the mule (42) and in the zebra (37-44) than in the horse (33-37), in *E. grevyi* (35-38) and in *E. ferus* (33-38). *E. hemionus* (35-43) and the quagga (36-41) do not fit in this scheme.

The cranio-frontal index expresses the relative width of the cranium. The range of variation of this index in the horse and in

the ass is exactly the same (46-54), and we also do not find this index characteristic for any other species.

The palatal angle, introduced by EWART (1907, p. 562), is measured as the distance from the middle of the posterior border of the palate to the line between the median incisor border and the foramen magnum. In some cases this line touches the posterior border of the palate (EWART, l.c.), in these cases (*E. caballus* no. 5; *E. hemionus* no. 5; *E. grevyi* no. 2) the value for the measurement is negative. Every species is very variable in this respect; it is remarkable that the value found in the mule (no. 1) is by far the largest of all.

The degree of deflection of the face is better expressed in the palato-cranial angle of OSBORN, measured between the basi-cranial line, taken outside, and the basifacial line, which passes through the median incisive border and the middle of the posterior border of the palate. But for the discrimination between the species this angle has no value, except that in *E. ferus* (12° - 19°) it is constantly smaller than in *E. hemionus* (19° - 33°). In the mule this angle (32°) is greater than in *E. caballus* or in *E. asinus* (up to 27°).

The occiput-vertex angle is greater in the horse (69° - 81°) than in the ass (56° - 69°). The mule (57°), the zebra (57° - 69°), *E. hemionus* (52° - 69°) and *E. grevyi* (64° - 69°) agree with the ass; only in *E. ferus* (59° - 76°) and in *E. quagga* (58° - 72°) this angle may become greater so as to agree with the horse. The smallness of this angle is an indication for the prominence of the occipital crest. OSBORN (1912, p. 58) states that nearly all horse skulls stand when set up on the (caudal) end, some mule skulls (one out of four), and no ass skulls. Of the twelve calvariums of *E. caballus* only one (no. 2, with the minimum value for the occiput-vertex angle, 69°) does not stand when set up on the caudal end. This is constantly the case in the other specimens examined by me, even in the calvariums of *E. ferus* and *E. quagga* which present an occiput-vertex angle greater than 69° .

The orbit is often relatively higher in the ass than in the horse (orbital index: *E. caballus* 79-93, *E. asinus* 88-105). The mule (83) resembles the horse, the hinny (92) more the ass. Like the ass are the zebra (87-107), *E. grevyi* (90-100) and the quagga (88-100). In *E. hemionus* (82-104) and in *E. ferus* (80-98) we find also lower values for this index, like in the horse.

FRANCK (1875, p. 41) was the first to indicate an important

point of distinction between horse and ass. He claims that in the ass the distance from the foramen magnum to the middle of the vomer incision is greater than that from the same point on the vomer to the middle of the posterior border of the palate; in the horse the latter distance is greater than the former. It can be seen from my table that in the horse FRANCK's vomer index indeed always is less than 100, but in the ass it is not always more than 100; in one skull of *E. asinus* (no. 2) the index is only 94. Such exceptions have also been found by other authors: in a skull of *E. asinus taeniopus* measured by NEHRING (1884, p. 98/99 and 111) the index is 91. ANTONIUS (1913, p. 247) states that a female skull of *E. asinus somaliensis* examined by him presents the horse-type of this index; from his table (l.c., p. 298, no. 19) this skull is seen to be normal (index is 106), but another ass in ANTONIUS's table (no. 5) has an index of 92. In *E. ferus* (74-99) FRANCK's index presents the horse-type, whereas for the mule (95-102), the zebra (87-127), *E. hemionus* (83-129), *E. grevyi* (86-102) and *E. quagga* (83-108) the index is not characteristic.

The length of the diastema often is relatively greater in the horse (diastema index: 19-24) than in the ass (15-21). The mule (20-23) and *E. grevyi* (19-23) agree with the horse, and the hinny (17), the zebra (18-21) and *E. hemionus* (14-20) with the ass. *E. ferus* (16-22) and *E. quagga* (15-25) may have a longer diastema, as in the horse.

The proportional width of the muzzle is a character of some value. In one of the skulls of *E. asinus somaliensis* of MOTOHASHI the muzzle index is 32, in the others and in my ass-skulls never more than 28. In *E. caballus* this index varies from 27 to 31. In the hinny (35), in *E. ferus* (30-37) and in *E. quagga* (30-39) the muzzle is relatively wider than in the horse, in *E. grevyi* (27-33) it is almost as broad, whereas in the zebra (24-36) and in *E. hemionus* (26-36) we may find both greater and smaller values for the muzzle index than in the horse.

The relative length of the tooth-series, as expressed in the dental index, is often greater in the ass (34-38) than in the horse (30-36); the hinny (36) and the mule (31) differ much from each other in this respect. In *E. grevyi* (30-34) and *E. quagga* (30-35) the tooth-series is relatively shorter than in *E. ferus* (34-39) and in the ass. In *E. zebra* (31-37) and in *E. hemionus* (32-40) the range of variation extends below that in *E. asinus*, in *E. hemionus* even above that in *E. ferus*.

No reliance can be placed on the molar index. This was to be expected, as it is known (GIDLEY, 1901, p. 99; SALENSKY, 1902, p. 52; DUERST, 1908, p. 412) that the relations of length and breadth of the teeth are not decisive.

The above review of the indices in the calvarium can be summarized as follows: the greatest craniometrical similarity to the horse is found in *E. ferus*, the next species is *E. grevyi* and then follows *E. quagga*. *E. hemionus* and *E. zebra*, on the other hand, display a greater craniometrical similarity to the ass than to the horse.

I shall proceed now with the description of the calvarium of *E. sivalensis* collected by DUBOIS.

Equus sivalensis Falconer et Cautley

Equus sivalensis FALCONER et CAUTLEY, Fauna Antiqua Sivalensis, London, 1849, pl. 81 figs. 1, 3-4, pl. 82 figs. 1, 3-6, pl. 84 figs. 1-4, pl. 85 figs. 1-8; MURRAY, The geogr. distr. Mamm., London, 1866, p. 331; FALCONER, Pal. Mem. **1**, London, 1868, p. 22, 186, 524; LYDEKKER, Rec. Geol. Surv. Ind., **9**, 1876, p. 90; MEDLICOTT and BLANFORD, Manual Geology India, part **2**, Calcutta, 1879, p. 574; LYDEKKER, Journ. As. Soc. Beng., **49**, part 2, 1880, p. 35, Mem. Geol. Surv. Ind., ser. 10, **2**, 1882, p. 87, pl. XIV figs. 1-2, pl. XV fig. 1; BRANCO, Pal. Abh., **1**, part 2, 1883, p. 27 (65); LYDEKKER, Rec. Geol. Surv. Ind., **16**, 1883, p. 83, 90, Mem. Geol. Surv. Ind., ser. 10, **3**, 1884, p. 131, Scient. Trans. Roy. Dublin Soc., ser. 2, **3**, 1884, p. 84, Cat. Siw. Vert. Indian Mus., part 1, Calcutta, 1885, p. 54; MAJOR, Quart. Journ. Geol. Soc., **41**, 1885, p. 3; KOKEN, Pal. Abh., **3**, part 2, 1885, p. 50; LYDEKKER, Cat. Foss. Mamm. Br. Mus., part 3, London, 1886, p. 66; PAVLOW, Bull. Soc. Imp. Nat. Moscou, n.s., **3**, 1889, p. 698; TROUESSART, Catalogus Mamm., nov. ed., **2**, Berlin, 1898, p. 792; WOODWARD, Outlines of Vertebrate Palaeontology, Cambridge, 1898, p. 338; SCHLOSSER, Abh. K. Bayer. Akad. Wiss., Math. - Phys. Kl., **22**, 1903, p. 199; TROUESSART, Catalogus Mamm., Quinquennale Suppl., Berlin, 1905, p. 644; RUTTEN, Die diluvialen Säugetiere der Niederlande, Berlin, 1909, p. 51; PILGRIM, Rec. Geol. Surv. Ind., **40**, 1910, p. 201; LYDEKKER, The horse and its relatives, London, 1912, p. 101, 247; ANTONIUS, Beitr. Paläont. Geol. Oest. Ung. u. d. Orients, **26**, 1913, p. 287; PILGRIM, Rec. Geol. Surv. Ind., **43**, 1913, p. 324; ANTONIUS, Grundzüge einer Stammesgeschichte der Haustiere, Jena, 1922, p. 265; SCHWARZ, Journ. Mamm., **3**, 1922, p. 151; MATTHEW, Bull. Am. Mus. Nat. Hist., **56**, 1929, p. 444, 461, 530; COLBERT, Trans. Amer. Phil. Soc. Philad., n.s., **26**, 1935, p. 160; HOPWOOD, Proc. Zool. Soc. London, 1936, p. 907; WADIA, Geology of India, London, 1939, p. 272; PILGRIM, Geol. Mag., **81**, 1944, p. 30.

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- Eq[uius] nomadicus* PAVLOW, Bull. Soc. Imp. Nat. Moscou, n.s., **3**, 1889, p. 701.
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- E[quus] palaeonius*, MEDLICOTT and BLANFORD, Manual Geology India, part 2, Calcutta, 1879, p. 574.

Equus & Hippotherium, FALCONER and CAUTLEY, Fauna Antiqua Sivalensis, London, 1849, pl. 83 figs. 1-3, 5-7, 9, 11.

Equus, FALCONER and CAUTLEY, Fauna Antiqua Sivalensis, London, 1849, pl. 84, figs. 13-14.

The calvarium from Jhil, Punjab, in the DUBOIS collection (no. 3222) belonged to an about five years old female, as inferred from the little worn M³ (stage VIII in OSBORN, 1912, p. 60), and the absence of C. The specimen is somewhat crushed. The incisor portion of the premaxillaries, and the free part of the nasals 1 cm in front of the naso-premaxillary notch are missing. The posterior portion of the cranium is badly damaged and displaced along a fractural plane which passes just above the occipital condyles, and which intersects the frontals somewhat behind the supraorbital process. It cannot be placed in its proper position without demolishing the specimen still further.

The preserved nasal processes of the premaxillaries are unsymmetrically shaped, the left is higher than the right. They expand a little about four cm in front of the lateral notch, just above the P². In side view, the naso-premaxillary suture is seen to run downward and then a little forward from the deepest point of the notch, so that the nasal is to some extent wedged in between the premaxillary and the maxillary.

The lateral surface of the maxillaries is concave in front of P². The outer styles of the latter tooth, and, in part, also those of P³, are exposed on the right side; this seems, however, to be a natural defect in the bone. In the molar region, the surface has broken off below the facial crest. But at the left side the ectolophs of the premolars are missing, consequently the left maxillary is much less complete than the right. The infraorbital foramen is situated above the posterior part of P⁴, about two cm below the naso-maxillary suture. The facial crest is incomplete on both sides. Above it, slightly in front of the orbit, there is a faint circular depression, the malar fossa.

The nasals are heavily depressed in the middle from the broken edge backward for about 7 cm of their length; this is clearly due to a lateral pressure, behind this region the median depression is only slight. The nasals remain of about equal breadth up to 80 mm behind the naso-premaxillary notch, then, within 30 mm of length, they expand from 65 mm width to 115 mm width, where they come into a contact with the lacrimals. More backward they expand only very little (to 122 mm at the posterior border of the



Fig. 1. Enamel pattern of right pd^1 - M^3 of *Equus sivalensis* Falconer et Cautley, Coll. DUBOIS, no. 3222. About natural size.

lacrimal), and the naso-maxillary suture is straight. The nasofrontal sutures are two arches, unfortunately not sharply defined; the frontals seem to indent the nasals sharply in the median line. The naso-maxillary suture on each side is much depressed at the point of expansion of the nasals; this depression, above and behind the infraorbital foramen, does not extend to the lacrimal. The position of the lacrimal tubercle unfortunately is not clear in the fossil.

The frontals are superficially damaged, their upper surface is flat. In the zygomatic process, entire at the right side, there are two supraorbital foramina. As the surface of the displaced cranial portion is injured, nothing definite can be said about the divergence and point of fusion of the frontal crests, and the shape of the nuchal crest. The orbit is longer than high, the borders of the left are incomplete. The posterior border projects laterally and forward.

The dentition is badly damaged. The pd^1 is present on both sides. What is preserved of the enamel patterns is shown in fig. 1.

LYDEKKER (1882, pp. 87-96) mentions the following characteristics of the fossil Siwalik and Narbada species of *Equus*: In *E. sivalensis* the premolars have comparatively short protocones, not longer than that in M^2 . The pd^1 is persistent, and larger than the corresponding tooth in the recent species. In the structure of the molars the fossil form is extremely close to *E. hemionus*,

in which, however, the teeth are smaller¹). The presence of a „larmial cavity” in the fossil form is noted, such a depression would be absent in the living species.

E. namadicus is said to differ from *E. sivalensis* in the greater length of the protocone both in the premolars and in the molars; this is illustrated by beautiful figures of the dentition (l.c., pl. XIV). Moreover LYDEKKER indicates a relation in the skull different for the two forms. He divides the length from M^3 to the foramen magnum by the total length pd^1-M^3 , and found 1.16 and 1.13 for *E. sivalensis* (close to *E. hemionus*, for which he gives 1.15), against 1.28 for *E. namadicus* (close to *E. caballus*, for which he gives 1.26 and 1.35).

Among the many authors who studied the cranial characters of the Equidae only two made observations on the fossil Indian species, viz., BRANCO (1883) and TSCHERSKI (1892). Their statements will be dealt with below.

Of the present calvarium, and of those figured by FALCONER and CAUTLEY (1849, pl. 81) the front part is missing, consequently we lack important data, such as the vertex, basilar, facial, and diastema length, the various indices based upon them, and the palatal, palato-cranial, and occiput-vertex angle. LYDEKKER (1882, p. 89) mentions that in the Indian Museum there is a Siwalik skull exhibiting the premaxillaries, but of this specimen he gives no further details than that the premaxillaries are intermediate in length between those of the domestic horse and the kiang, but approach nearer to those of the latter, and that the diastema I^3-P^2 is very much shorter than in *E. caballus*.

BRANCO (1883, p. 21) studied a plaster-cast of the calvarium of *E. namadicus* figured by FALCONER and CAUTLEY (1849, pl. 81 fig. 5). He measured the length from the occipital crest to the anterior border of P^2 , and found that in relation to the frontal width the calvarium of *E. namadicus* is longer than in *E. caballus* and in *E. asinus*. In my fossil specimen the two measurements used by BRANCO cannot be taken with sufficient accuracy. In six skulls of

¹) Originally LYDEKKER (1882, p. 92) regarded *E. hemionus* as not improbably the descendant of *E. sivalensis*. Afterwards (LYDEKKER, 1912, p. 249) he withdraws this suggestion, because the teeth of the kiang are „so much smaller” than those of *E. sivalensis*. The Arab horse, if specifically distinct from *E. caballus*, is now suggested to have originated from the Siwalik species, probably through *E. namadicus* (see also, l.c., p. 163/64).

E. hemionus, and in four of *E. ferus* this ratio proves to be fairly constant, the length from the occipital crest to P² is proportionally

	<i>Equus hemionus</i> Pallas						<i>E. ferus</i> Pallas				„ <i>E. namadicus</i> ” (Branco)
	1	2	3	4	5	6	1	2	3	4	
1. Frontal width	198	207	201	199	205	211	199	205	223	225	ca. 195
2. Length from occipital crest to anterior border of P ²	388	402	382	387	405	411	392	402	412	414	445
3. Ratio 1 : 2	0.51	0.51	0.53	0.51	0.51	0.51	0.51	0.51	0.54	0.54	0.44

less than in the Siwalik species. But, as BRANCO (l.c., p. 22/23) correctly states, this does not imply that also the whole skull of *E. namadicus* is relatively longer, since no allowance is made for the length of the diastema, which affords a decisive character (above p. 252). I mentioned already that LYDEKKER found the diastema in *E. sivalensis* to be very much shorter than in *E. caballus*.

The naso-premaxillary suture in the present fossil calvarium is different from that in the recent species. In every recent skull examined by me in which the sutures are shown, the premaxillary is wedged in between the nasal and the maxillary, and extends distinctly behind the lateral notch. According to PETIT (1937, p. 29) the position of the naso-premaxillary suture affords a good character to distinguish between horse and ass. In the horse the deepest point of the notch is formed by the nasal, whereas in the ass the premaxillary encroaches higher on the sides, and forms the deepest point of the notch. I agree with PETIT as to the validity of this character, though the difference sometimes is only very slight. *E. hemionus* (fig. 2 a and b) presents the horse-type of the naso-premaxillary suture, and so does our fossil (fig. 2c); the ass-type is shown in fig. 2d. In our fossil the premaxillary does not extend so far behind the notch as in the recent species, and a narrow tongue of the nasal, wedged in between the premaxillary and the maxillary, extends in advance of the notch, which as far as my observations go, is never the case in the recent Equidae.

The position of the infraorbital foramen has no value for purposes of discrimination, I agree with CHUBB (in: OSBORN, 1912, p. 89) and ANTONIUS (1913, p. 249) in this respect. Though in four skulls of *E. ferus* its upper margin is nearer to the naso-maxillary suture (from 18 to 23 mm) than in five of the six skulls of *E. hemionus* (from 27 to 32 mm), in the sixth *hemionus*-skull

(no. 3) the distance is not greater (20 mm at the right, and 23 mm at the left side).

The „larmial cavity” of the description of LYDEKKER (1882, p. 90), afterwards (LYDEKKER, 1904, p. 426; 1912, p. 22) called the preorbital pit or depression, is the lower or malar fossa (GREGORY, 1920, p. 269). It may occur in the recent species too (*E. caballus*, nos. 2 and 6; *E. asinus*, nos. 4 and 5; *E. hemionus*, nos. 5

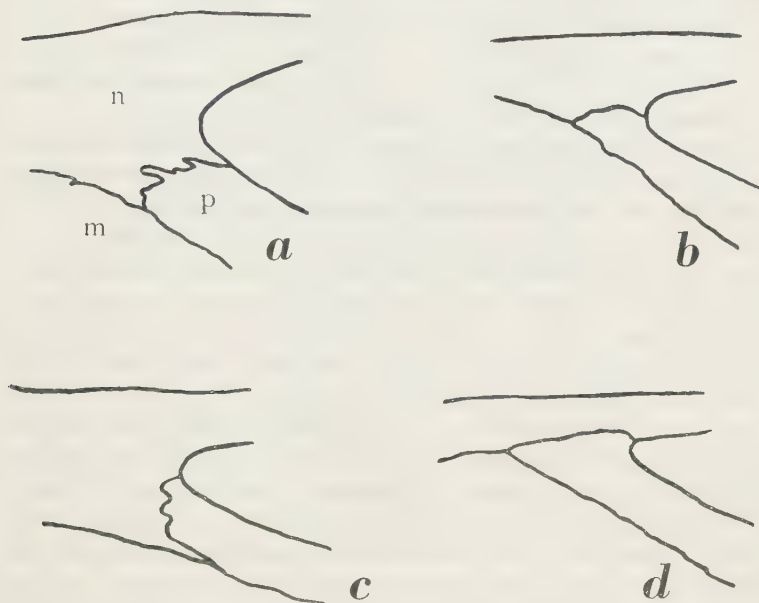


Fig. 2. Naso-premaxillary suture, right view. a-b, *Equus hemionus* Pallas; a, Leiden Museum, cat. a; b, Amsterdam Museum, no. 524; c, *Equus sivalensis* Falconer et Cautley, Coll. DUBOIS, no. 3222; d, *Equus asinus* L., Leiden Museum, cat. e. n, nasal; p, premaxillary; m, maxillary. Two-thirds natural size.

and 6; *E. ferus*, nos. 1 and 2; *E. quagga quagga*, no. 1, and *E. quagga chapmani*, no. 6).

The deep median depression of the nasals in our specimen is also shown in the specimens figured by FALCONER and CAUTLEY (1849, pl. 81). According to TSCHERSKI (1892, p. 338), who studied plaster-casts of the latter calvariums, this is the result of a lateral pressure, like it is also in our specimen. The posterior part of the nasals, not subjected to the crushing force, has only a slight depression in the median line. According to SALENSKY (1902, p. 31)

the posterior part of the nasals offers decisive characters. He claims that in the horse the nasals attain their greatest width at the posterior end of the naso-lacrimal suture, which latter is straight. In the ass the greatest width of the nasals is found at the anterior end of the naso-lacrimal suture, and the lacrimal border is concave. As appears from the above description, our fossil is perfectly in accord with *E. caballus*. This character, however, does not hold in all cases (DUERST, 1908, p. 409; ANTONIUS, 1913, p. 248); in a skull of *E. asinus* (no. 4) I found the naso-lacrimal suture to be straight, and the nasals wider at its posterior than at its anterior end. SALENSKY (l.c.) states further, that in the shape of the nasals *E. hemionus* resembles the ass, and *E. ferus* the horse, which is in accordance with my own observations. In one skull of *Equus hemionus* (no. 4) the lacrimal border is concavo-convex from before backward, but the width of the nasals is greater at the anterior end of the naso-lacrimal suture than at its posterior end.

The shape of the sutures between the nasals and the frontals is considered as characteristic. SALENSKY (1902, p. 32) states that in the horse these sutures are fairly high arches, whereas in the ass they are less arched, and in some cases even almost straight. To express this difference in numbers, OSBORN (1912, p. 86) introduced a new measurement, viz., the distance from the posterior end of the internasal suture to the middle of the line connecting the two most posterior points of the naso-frontal suture. From my table (Convexity of naso-frontal suture) it is evident, that, though useful, this character cannot be fully relied upon; in *E. caballus* this measurement varies from 26 to 42 mm, in *E. asinus* from 12 to 35 mm. DUERST (1908, p. 409) and ANTONIUS (1913, p. 247) allude to the great individual variability in the shape of the naso-frontal suture. MOTOHASHI (1930, p. 26) states that in *E. hemionus* the convexity of the naso-frontal suture is greater than in the other species, but in one of my skulls it is only 22 mm. CHUBB (in: OSBORN, 1912, p. 89) observed that the shape of the nasals is not characteristic for the Asiatic asses.

The position of the lacrimal tubercle is very characteristic. ARLOING (vide PETIT, 1937, p. 24) observed that in the ass it is situated on the naso-lacrimal suture, and PETIT (l.c.) regards it as an excellent character, perhaps the best. Unfortunately this tubercle often is ill-defined. In *E. caballus* this tubercle is always distinctly apart from the nasal border, in one skull (no. 7) it is even nearer to the orbital margin than to the nasal. The skull of

the mule in the Leiden Museum resembles the horse in this respect. In *E. asinus* this tubercle is situated on the naso-lacrimal suture with two exceptions (nos. 4 and 6) in which it is placed medially of that suture, thus on the nasal!

For the other species the position of this tubercle is also characteristic, as far as my observations go. In *E. ferus* the position is like that in the horse, in *E. hemionus*, *E. grevyi* and *E. quagga* it agrees with that in the ass. In one skull of *E. hemionus* (no. 3) the lacrimal tubercle is exceptionally prominent, in a skull of *E. quagga chapmani* (no. 8) and in one of *E. quagga granti* (no. 10) it is situated on the nasal.

MOTOHASHI (1930, p. 32) considers the presence of more than one supraorbital foramen on each side as very markedly characteristic of typical *E. hemionus* from Western Mongolia. In general in recent horses there is one supraorbital foramen on each side; at one side it may be duplicated, or there may be several, usually very small, additional perforations close to the orbital margin. I counted three or four foramina on each side in one skull of *E. hemionus* (no. 3), but also in *E. caballus* (no. 5), *E. quagga burchellii* (no. 5) and in *E. q. chapmani* (no. 8); at one side only in *E. hemionus* (nos. 1 and 2), but also in *E. quagga quagga* (no. 1) and in *E. q. burchellii* (nos. 3 and 4).

The orbital index in our fossil specimen is 81, slightly less than in any of the twenty-eight skulls of *E. hemionus* (from 82 to 104), but still within the range of variation in *E. caballus* (79-93). In the two specimens represented by FALCONER and CAUTLEY (1849, pl. 81 figs. 1 and 5) the orbit seems to be even more elongated¹). According to the measurements in FALCONER (1868, pp. 524-527) given in mm in the table II below, the orbital index is 73 in the first, and only 65 in the other; values not met with in recent horses. BRANCO (1883, p. 28) who examined a plaster-cast of the calvarium represented on pl. 81 fig. 5 of FALCONER and CAUTLEY (1849) writes that the orbit is strikingly long, much different from that in the other species of *Equus*²). It seems, however, from the figure that part of the posterior border of the orbit is missing; it

¹) Fig. 5a of pl. 81 of FALCONER and CAUTLEY (1849) must have been reversed, since it represents the right zygomatic arch which is wanting in the other aspects of the same specimen.

²) BRANCO gives the vertical and horizontal diameter of the orbit as 49 and 80 mm respectively, which makes the index not more than 61.

projects less laterally and forwards than in the calvarium of fig. 1. This may very probably account for the extremely elongated shape of the orbit in this specimen.

According to the measurements given by BRANCO (1883) the vomer index in the calvarium of *E. namadicus* is 97 (BRANCO, l.c., p. 23 gives 93), this is a value about intermediate between horse and ass.

In our specimen the length of the tooth-series exceeds the distance between M^3 and the foramen magnum. LYDEKKER (see above, p. 257) placed reliance on the ratio of these two measurements for discrimination between *E. sivalensis* and *E. namadicus*. This ratio (I divide the length P^2-M^3 by the distance between M^3 and the foramen magnum) is greatly influenced by age, since during life the length of the tooth-series grows shorter. Even in three calvariums of *E. hemionus* I found such different ratios, that the valuelessness of this ratio is self-evident. It is easy to find skulls of *E. caballus* or of *E. zebra* in which the ratios differ more

	Coll. DUB. no. 3222	LYDEKKER, 1882 p. 90									
		<i>siva-</i> <i>lensis</i>	<i>nama-</i> <i>dicus</i>	2	4	5	5	6	1	2	
1. Length P^2-M^3	196	—	—	—	158	154	150	190	160	153	126
2. Distance M^3 to foramen magnum	190	—	—	—	185	171	201	209	223	153	178
3. Ratio 1 : 2	1.03	0.86	0.88	0.78	0.85	0.90	0.75	0.91	0.72	1.00	0.71

from each other than they do in *E. sivalensis* and *E. namadicus*. In one skull of the zebra (no. 1), in which, like in our fossil, the M^3 is but little worn, the length P^2-M^3 is equal to the distance from M^3 to the foramen magnum. That the tooth-series in our calvarium is relatively longer than in LYDEKKER's specimens is certainly due to the younger age of our specimen.

In table II I give the measurements of our specimen, to be compared with those of the calvariums and upper teeth figured by FALCONER and CAUTLEY (1849) and given (in inches) in FALCONER (1868, pp. 524-527). Some of them are identified as *E. namadicus*, in these cases I placed the latter name within parentheses at the head of the column. The measurements of the teeth figured by LYDEKKER (1882, pl. XIV) are taken from the figures.

It can be seen that the fossil specimens often present greater

dimensions than *E. hemionus*. The posterior portion of a calvarium represented by FALCONER and CAUTLEY (1849, pl. 81 fig. 2) is remarkable for its large occiput and narrow cranium; whether it can be referred to the same species as the other specimens figured in the same plate seems doubtful.

The range of variation of the teeth in five skulls of *E. hemionus* measured by TSCHERSKI (1892, p. 360/63) and that in the seventeen skulls of which MOTOHASHI (1930, table 15) has given the measurements, are greater than that in my material. Finally I added the tooth-measurements of a recent, and of the Pleistocene skull from Sjara-osso-gol, Ordos, N. China, given by BOULE and TEILHARD (1928, p. 42)¹). In the latter specimen, of which measurements have already been given under the head of *E. hemionus* as no. 7 in table I, the P⁴ and M² are slightly longer, and the P² and M³ somewhat broader than the corresponding teeth in a series of twenty-nine recent skulls.

Only a small majority of the teeth of the Siwalik and Narbada specimens is larger than those in *E. hemionus*. In the teeth referred to *E. namadicus* and represented in fig. 3 of pl. XIV of LYDEKKER (1882), which completely fall within the range of variation of *E. hemionus*, the protocones are longer. The teeth of fig. 2 of the same plate, referred to *E. sivalensis*, are larger than those of *E. hemionus* (except for the lengths of P⁴, M¹ and M²), but the protocones are not longer. The different lengths of the protocones LYDEKKER considers as characteristic; in *E. namadicus* the protocones of P³ and P⁴ are longer, in *E. sivalensis* shorter than that of the M² of the same series. In our fossil the premolar-protocones are intermediate in length between those of *E. sivalensis* and *E. namadicus* figured by LYDEKKER (1882, pl. XIV figs. 2-3), the protocone of M¹ is as long as in the latter, and that of M² has the same length as in the former. The teeth of fig. 1 of LYDEKKER's plate, referred to *E. sivalensis*, have longer protocones in the molars than those of fig. 2 of the same plate. In the other permanent tooth-series figured by LYDEKKER (1882, pl. XV figs. 3-4) the M² is not represented, but LYDEKKER refers them to *E. namadicus* on the ground of the relatively large size of the protocones in the premolars.

In my fossil specimen the length of the protocone in P³ and P⁴

¹) The breadth of P² and M¹ in the former specimen is given as 34 mm, evidently a misprint for 24 mm.

exceeds that of the protocone in M^2 , consequently it would be referred to *E. namadicus*. The variation in *E. hemionus* and other species makes it evident, however, that this character is not decisive. Firstly there is seen to be a great variation in the length of the protocone in every tooth of the series. GIDLEY (1901, p. 103) writes that, though "the antero-posterior diameter of the protocone is very little affected by wear the range of individual variability of this diameter is very great, and cannot be depended upon as a distinguishing character even in corresponding teeth of individuals of the same size". And secondly, the criterion used by LYDEKKER proves to be not constant within a species. Though in *E. hemionus* most often the length of the protocones of P^3 and P^4 , or only that of P^3 , is shorter than that of M^2 , in some specimens measured by MOTOHASHI (1930, table 15, A.M. 57204 and A.M. 57206) the protocones of P^3 and P^4 are longer than that of M^2 . The same variation occurs in *E. ferus* (MOTOHASHI, l.c., A.M. 70215 and A.M. 35004) and in *E. asinus somaliensis* (l.c., F.C.M. 0-473 and F.C.M. 0-352). In four skulls of *E. ferus* two have the protocones of P^3 and P^4 shorter, and the other two longer than that of M^2 ; *E. asinus* may vary in the same way.

Length of protocone in	<i>E. ferus</i> Pallas				<i>E. asinus</i> L.	
	1	2	3	4	3	5
P^3	14	13½	12	17	8½	10
P^4	13½	15	12½	16	8½	10
M^2	13	15	13½	15	9	9

The union of *E. sivalensis* and *E. namadicus* into one species, already alluded to by MATTHEW and COLBERT, thus seems to meet no further objection, and the species may be known under the name *Equus sivalensis* Falconer et Cautley.

TABLE I. Measurements, indices and angles of the skull in the recent species of *Equus* and in some of their hybrids.

TABLE II. Comparative measurements of calvarium and upper teeth of *Equus sivalensis* Falconer et Cautley and *Equus hemionus* Pallas

				<i>Equus sivalensis</i> F. & C.													
				Coll. Dubois 3222 FALCONER, 1868, PP. 524-527 Fauna Ant. Siv., pl. 81-181-281-5 (<i>nam.</i>)		<i>Equus hemionus</i> Pallas											

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Equus sivalensis Falconer et Cautley. Calvarium from Jhil, Punjab; DUBOIS collection no. 3222. Left figure, upper view; right figure, right view
 $\frac{1}{3}$ natural size.

THE STRUCTURE AND THE MECHANISM OF MOVEMENT OF THE GILL-FILAMENTS IN TELEOSTEI

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1. *Introduction.*

The few communications on the detailed structure of the gills, which have been published in the course of years, are mostly of older date. In connection with the more recent investigations on the respiration of *Teleostei* (VAN DAM 1938, HAZELHOFF 1938) it seemed useful to study once more the apparatus of support and of movement of the gill-filaments (gill-plates of the first order), which are arranged in a double row, the two hemibranchiae, on each visceral bar.

Every gill-filament is supported by a piece of skeleton, the gill-rod, which is connected with the visceral arch. The filaments are movable through the action of small muscles. They bear the lamellae or gill-plates of the second order. The exchange of carbonic acid and of oxygen takes place in the blood-lacunae of these lamellae. The physiological investigations mentioned above have revealed, that the position of the filaments during the respiration is a different one to those found in most of the text-books. The figures of a horizontal section of the branchial region in a *Teleost* e.g. in GEGENBAUER II, 1901, p. 226; BOAS 1922, p. 511; HERTWIG 1931, p. 526; CLAUS-GROBBEN-KÜHN 1932, p. 919; NEAL and RAND 1939, p. 247; PARKER and HASWELL II 1943, p. 86, show a wide aperture between the filament-tips of the posterior hemibranchia of one gill and the anterior one of its immediate successor (fig. 1a). If there should be such an aperture between every two successive gills, the greater part of the respiratory water would flow away without having passed the lamellae. The value of the utilisation of oxygen (the difference in quantity of oxygen of the water before and after passing the gills) must be low under these conditions. On the contrary it appears, that the value of the utilisation of oxygen in *Teleostei* is particularly high. In the eel and

in the trout VAN DAM (1938) found a utilisation of 80%; according to HAZELHOFF this value amounts to an average of 62% in some other *Teleostei*. VAN DAM rightly concluded from this, that the separation between the spaces medial and lateral of the gill-elements, which are respectively called inspiratory and expiratory cavity, must be a wall without big slits. The gill-filaments with their lamellae must form a continuous wall between the inspiratory and the expiratory cavity. Only in this case all the respiratory

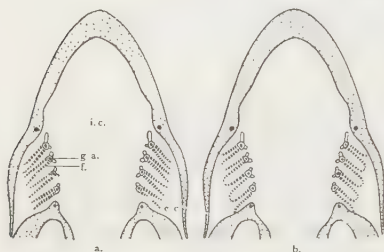


Fig. 1. Schematic diagram of a horizontal section of the head of an osseous fish. a. Wrong reproduction of the position of the filaments during quiet respiration (after BOAS 1922). b. Correct reproduction of the position of the filaments during quiet respiration. g.a.: gill-arch; f.: filament with lamellae; i.c.: inspiratory cavity; e.c.: expiratory cavity.

water will pass through the small slits between the lamellae, and will come into close contact with the respiratory epithelium (fig. 1b). In his figures 20 a and b (1938) VAN DAM gives a schematic diagram of this separating wall between the inspiratory and the expiratory cavity. The filament-tips of the successive hemibranchiae of two different branchial arches touch each other.

As early as 1932 WOSKOBOINIKOFF found, that in living *Teleostei* the filaments of two successive branchial arches shut off the slits between these arches. "Auf diese Weise," Wos-

KOBOINIKOFF says on p. 328, "entsteht eine besondere Einrichtung zur maximalsten Berührung des Wassers mit dem Blutsystem der Blättchen".

RAUTHER (1937, p. 226, fig. 200b) copies the figure of WOSKOBOINIKOFF in his summary on the structure of the gills of *Anamnia*.

In perfect agreement with the above mentioned conclusions are the results of experiments on *Tinca tinca* by Mrs. HOFDIJK-ENKLAAR, carried out in the Zoological Laboratory of the University of Groningen under the direction of Prof. HAZELHOFF (not published). As the gill-cover is raised for a moment in every expiration, it is possible to watch the position of the filament-tips by looking under the gill-cover in this phase of breathing. Mrs. HOFDIJK-ENKLAAR found, that in this phase of the respiration the filament-tips of one hemibranchia and those of the successive one

of a following branchial arch lean against each other. So during quiet respiration the aperture between two successive gills is closed in expiration. As during inspiration the edge of the gill-cover lies against the body, the position of the filaments is absolutely hidden from view in this phase of breathing. It was necessary to observe the position of the filaments during both phases of the respiration. To attain this Mrs. HOFDIJK-ENKLAAR made a small circular opening in the gill-cover and inserted a bit of transparant celluloid in it. The pressure-relations in the inspiratory and the expiratory cavity did not change by this. Through the transparant piece of celluloid Mrs. HOFDIJK-ENKLAAR could observe, that in quiet respiration the filament-tips of one hemibranchia and those of the successive one of a following branchial arch touch each other during inspiration as well as during expiration.

When the gill-cover was lifted up to watch the position of the filaments for some time, it was observed, that in every expiration the gill-clefts were opened for a moment. The insufficient closure of the expiratory cavity causes these movements of the filaments. It is obvious, that the raising of the gill-cover brings about an abnormal high difference in pressure between inspiratory and expiratory cavity. By this, during expiration, the filaments of two successive gills, bridging the gill-slit, will part for a moment, to recover their former position immediately.

In apparently clean water a normally breathing fish makes so-called coughing movements at regular intervals, e.g. 8 times to 50 breathing movements. The coughing movements become more frequent, if small fragments of defilement (small filaments of filtering-paper, a large quantity of detritus, etc.) are added to the water. After a short time these particles are coughed out either in forward direction and reappear in the mouth, or they are coughed out in backward direction and appear under the gill-cover. In his investigations concerning the respiration of the *Teleostei* KUIPER (1906, 1907) observed these abnormal respiratory movements, which he rightly interpreted as coughing movements. In his detailed study (1907) he accurately describes the sequence of opening and closing of mouth and gill-cover during the coughing movements. Mrs. HOFDIJK-ENKLAAR found, that during these coughing movements the gill-clefts open for a moment by the adduction of the filaments of the two hemibranchiae belonging to the same branchial arch. The coughing movement in forward direction is more vigorous than that in backward direction. The filaments,

bridging the gill-clefts, do not part from each other passively, but are moved by muscle-action.

Mechanical stimulation of the mucous membrane of the inspiratory cavity as well as of the expiratory one causes these coughing movements. The situation of the area, which is stimulated, determines, whether the coughing movement will take place in forward or in backward direction. Mechanical stimulation of the mucous membrane of the mouth causes coughing in forward direction, whereas stimulation of the inspiratory side of the filaments or of the mucous membrane of the respiratory cavity causes coughing movements in backward direction. By stimulating one filament or a few filaments, only an adduction of the stimulated filaments and of the adjacent ones takes place.

Afterwards I found that exactly the same adduction-movements could be called forth in stimulating the filaments of a freshly decapitated fish with an electric current. In such a test object the position of the gill-filaments is still the same as in the living animal. It was even possible to study the movements in the transverse section of the gills. In this manner a better insight could be obtained in the movement of the filaments. This is of importance in connection with the structure of the filaments.

As soon as we decapitate a fish and bring it in a fixation-liquid the filaments bridging the gill-cleft give up their position, so that a wide communication appears between the inspiratory and the expiratory cavity. Then the filaments attain the position, which is represented in the just mentioned textbooks. Obviously the figures of the gill-region in these text-books are derived from dead and fixed material.

Of late years no detailed original descriptions of the microscopical structure of the gill-filaments have appeared. Out of an anatomical point of view it seemed desirable to study once more the structure of those elements, which support, fix and move the filaments in connection with the altered views about the normal position and about the movements of the filaments in the living animal. The anatomical data of the following investigations are obtained from microscopical examination of gills of different fishes, which were cut in serial sections and from the dissecting of the gills under the binocular microscope.

2. Summary of the literature about the structure of the gills.

Before the middle of the last century already a few investigators

examined the structure of the gill-filaments of fishes. These investigations dealt especially with the small muscles, which move the filaments. The data were obtained from the dissection of gills, for which purpose only big representatives could be used. After WAHLBAUM, ALESSANDRINI, LEREBUILLET, BAZIN in this way had stated the presence of muscles in the spaces between the filaments, DUVERNOY gave a more detailed description of these muscle-elements in cartilaginous and bony fishes. I leave the cartilaginous fishes out of consideration, as they have not been

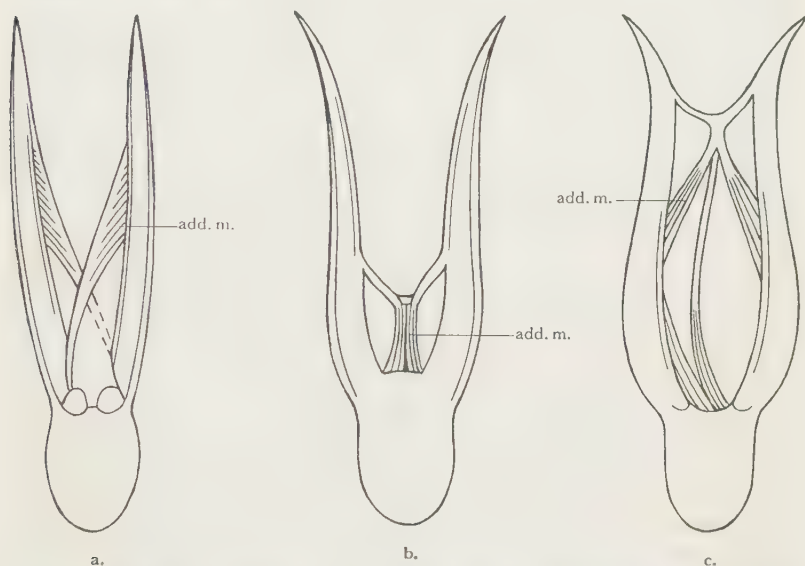


Fig. 2. Schematic diagram of the three types of adductor muscles after DUVERNOY. add.m.: adductor muscle.

investigated. In the bony fishes DUVERNOY (1839) distinguished three types of muscles in the spaces between the filaments. In a more or less schematic way we can reproduce them as in fig. 2. Whereas in type one and two the muscles should be absolutely independent of each other, in those of type three the muscles of two opposite filaments should join into one tendon, which should split again into two thin ones for the just mentioned filaments. DUVERNOY calls these muscles adductor muscles, that is to say, they should only bend the free extremities of the opposite filaments of both hemibranchiae of a gill to each other.

IN 1881 RIESS was the first, who gave a description of the microscopical structure of the filaments in *Teleostei*. Though the description of RIESS is not quite correct in every detail, his conceptions are copied without criticism even in more recent literature. RIESS has distinguished two types of adductor muscles. His first type (*Esox*, *Perca fluviatilis*) corresponds with type one of DUVERNOY; in his second type (*Salmo*, *Cyprinoidea*) he joins type two and three of DUVERNOY. After his description the adductor muscle of the first type originates from the base of two successive gill-rods, which support the filaments, and is fixed at a gill-rod of the opposite hemibranchia of the same gill about half way down. The contractions of these muscles cause every filament to pivot round an axis going through the basal part of the gill-rod parallel to the visceral arch.

The muscles of type two however should move the filaments in a quite different and even surprising way. RIESS changes the origin and the insertion of the muscles. The muscles were to take their origin from a ligament lying between the two hemibranchiae of a gill about half-way the length of the filaments, whereas they should have their insertion into the gill-rods nearer to the base of the filaments. They should pivot the filaments round an axis going through this ligament. In the following way he describes the function of these muscles (1881, p. 528): "Diese Muskeln ziehen als Musculi adductores die Basalhälften der gegenüberliegenden Kiemenblätter einander an, während sich die Spitzen derselben entfernen." If this should be true, the function of these muscles would be opposite to that of the muscles of the first type. The muscles should not act as adductors for the filament-tips, on the contrary, the distance between the filament-tips of both hemibranchiae of a gill should be increasing instead of decreasing. It is incomprehensible, that these two muscle-types should have an opposite function with regard to the filaments. Afterwards it will be shown, that this conception of RIESS is wrong. Moreover the movement of the filament in the way mentioned above should bring about a displacement of the base of every filament in regard of the visceral arch. The question whether the fibrous tissue, which connects the filament-rod with the visceral arch, allows this displacement is ignored.

In every fish investigated by RIESS thin muscle-bundles, which he called abductor muscles, were found at the base of the filaments of the oral hemibranchia in every gill. They have their origin on

the oral side of the visceral arch and their insertion on the basal extremity of the gill-rods of the filaments. As their name indicates, their action is opposite to that of the adductor muscles. So the movement of the filaments in fishes of the first and of the second type as distinguished by RIESS, should be different, as according to him the adductor muscles act in a different way in these types.

The broadened basal extremities of the gill-rods, indicated as "Gelenköhren" and "Gelenkköpfe" are mutually joined and are connected with the visceral arch by fibrous tissue, according to RIESS. As there is no space separating the surfaces opposite one another, it is better not to speak of a "Gelenk" in this case.

The action of the abductor muscles in *Esox* and in *Perca fluviatilis* (mm. add. of the first type) is supposed to be supported by a ligament, which connects the basal extremities of the gill-rods of both hemibranchiae belonging to one gill in the shape of a thin layer of elastic tissue. The contraction of the adductor muscles should strain the ligament, whereas after relaxation of these muscles the tension of the ligament should bring the filaments in their normal position again.

In contradiction to RIESS, OPPEL (1905) pointed out that the above mentioned ligament should support the adductor muscles in their function.

Without going into details RAUTHER (1937) states in a more recent short summary on the structure of the gills, that there exists a close contact between the filaments of the successive hemibranchiae of every two gills. This contact should be attributed to the special construction of the gill-skeleton, the muscles and the ligaments. Not a single detail is mentioned here. He distinguishes adductor and abductor muscles. Only the muscles of *Esox* (type one) are described. In his description of the muscles of the *Syngnathidae* RAUTHER (1925) follows RIESS.

It is evident, that the authors, where they speak of the significance of the muscles of the filaments, have drawn their conclusions from the anatomical data. They did not study the movements of the filaments in the living animal. We will discuss this significance of the muscles with regard to the movements of the filaments in the last chapter.

3. *Materials and methods.*

The gills of the following representatives of the *Teleostei* were examined:

Esox lucius (Esocidae) •
Umbra krameri (Umbridae)
Cobitis barbatula (Cobitidae)
Ameiurus nebulosus (Siluridae)
Gasterosteus aculeatus (Gasterosteidae)
Perca fluviatilis (Percidae)
Tinca tinca
Leuciscus rutilus }
Cyprinus carpio } (Cyprinidae)
Phoxinus laevis }
Rhodeus amarus }
Salmo irideus (Salmonidae)

In the beginning the animals were decapitated in a deep anaesthesia with aethyl urethane. This method was abandoned, for this anaesthesia caused an overfilling of the bloodvessels of the gills. Frequently even a haemorrhage appeared, which destroyed the normal connection between the epithelium and the underlying tissue. In future the small specimina were killed by destroying the brains with a needle, which was brought into the skull through the foramen occipitale. Before carrying out this manipulations in bigger specimina the brains were cut from the spinal cord with the aid of a pair of scissors.

Those gills, which should serve for microscopical examination, were fixed in the liquid of BOUIN, those which should be used for dissection, were brought into formaline 10%. The liquid of BOUIN gave a good fixation for our purpose, though frequently a shriveling was observed in the delicate tissues. Consequently it was difficult now and then to distinguish the way of attachment of the muscles to the gill-rods accurately. It was for this purpose, that gills of bigger specimina were dissected in a fixed as well as in a fresh condition. Moreover by this method the possibility of movement of the connections between the gill-rods and the gill-arch could be judged.

For the microscopical examination small pieces of gills were embedded in paraffin and cut in series (10 μ thick) in three directions perpendiculair to each other. As a rule the sections were stained with haematoxyline and eosine. By the staining method of WEIGERT for elastic tissue and afterwards by using orceine the distribution of the elastic tissue in the gills was investigated.

4. *The apparatus of support in the gills.*

The filaments or gill-plates of the first order, flattened sideways, are arranged on the epi- and ceratobranchialia of the visceral arches, which support the gills (fig. 3). The filaments of both hemibranchiae belonging to one gill are alternately placed (fig. 4). At their base these pairs of hemibranchiae are united with each other by a mass of tissue, called "diaphragme branchial" by DUVERNOY (1805). The afferent and efferent blood-vessels for the gills, the muscles for moving the filaments, etc. lie in this diaphragm. According to the length of the diaphragm we classify the

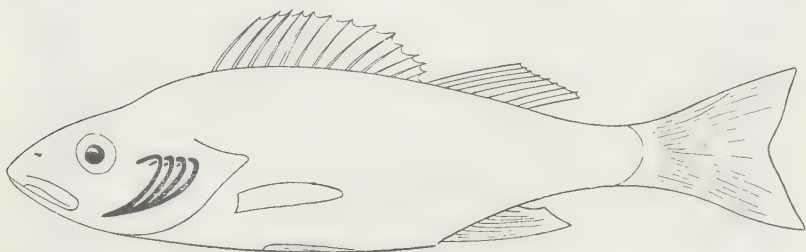


Fig. 3. Diagram showing the position of the left gill-arches (without gill-filaments) in the perch. Gill-cover represented as transparent. (After v. DAM, 1938.)

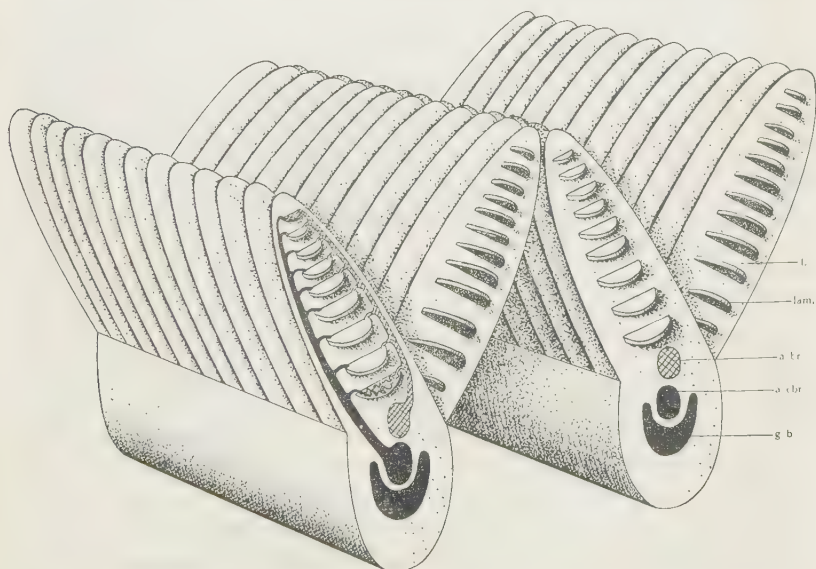


Fig. 4. Schematic diagram of the position of the filaments and the lamellae in the living animal showing the blood-circulation in the filament. f.: filament; lam.: lamella; g.b.: gill-bar; a.br.: arteria branchialis; a.ebr.: arteria epi-branchialis.

fishes investigated here into two groups. In *Esox lucius*, *Gasterosteus aculeatus*, *Cobitis barbatula*, *Ameiurus nebulosus* and *Perca fluviatilis* the length of the diaphragm amounts to $\frac{1}{4}$ or $\frac{1}{3}$ of the length of the filament, whereas in the *Cyprinidae* mentioned above and in *Salmo irideus* the diaphragm reaches up to half way the length of the filaments and higher. The thin lamellae or gill-plates of the second order, which contain blood-lacunae and which are covered with the respiratory epithelium, are ridges of the filaments standing nearly perpendicular on both sides of them without any supporting apparatus.

Each filament is supported by a barlike piece of skeleton, the gill-rod, which is connected with the visceral bar. The gill-rod has an excentrical position, viz., it lies in that part of the filament, which is turned towards the expiratory cavity (plate X fig. 1). In some species the gill-rods are straight, in others they are slightly curved towards the side of the inspiratory cavity. In *Salmo irideus* the gill-rod is lined with little teeth.

The gill-rod consists of a tissue of chondroid character (plate X, fig. 1). The large puffed up cells have the appearance of cartilage-cells and can be stained like them. The intercellular substance, however, is reduced to a thin layer, which only forms a framework between the cells. To the surface of the gill-rod the intercellular substance increases and is slightly calcified as a rule. Remnants of the cartilage-cells are found in this calcified layer. From this histological structure follows, that the gill-rod is rather rigid; to some extent however it is still flexible. It is easy to establish the flexibility of the filaments in bigger specimina. This property agrees with the functional exigences, which are demanded specially for the filaments of the *Cyprinidae* and *Salmo irideus*, as will be shown afterwards.

The transverse section of the gill-rod is sometimes nearly round, but mostly elliptical with the greatest divergence towards the direction, from which the water runs from the inspiratory cavity into the expiratory one (plate X fig. 1). In a number of fishes the gill-rods have crests or ridges, which increase the dimension in the same direction.

The bases of the rods are more or less broadened (plate X fig. 2). These basal extremities are connected with each other by a thin layer of connective tissue. So the filaments of every hemibranchia as a rule form a solid unit. This syndesmotomic connection brings about, that every gill-rod can only make a small movement with regard to his neighbouring ones.

The broadened basal part of the gill-rod, which is joined with the gill-bar, has the form of a pulley with the axis parallel to the gill-bar. The efferent bloodvessel of every filament, which runs on the inspiratory side of the filament, takes its way through the groove of the pulley (plate X fig. 2, plate XI fig. 1) to reach the arteria epibranchialis, which generally lies protected in an excavation of the gill-bar.

The connections between the gill-bar and the gill-rods lack a joint-cavity (plate XI fig. 2). For this reason it is better to avoid here the word articulation (see RIESS, p. 522 „Gelenkköpfe“, „Gelenköhren“) and to speak of a syndesmosis. The joining connective tissue is continuous with that of the syndesmosis of every two successive gill-rods.

In *Esox lucius* and *Gasterosteus aculeatus* a ligament consisting of elastic tissue (stainable after the method of WEIGERT for elastic tissue, plate XII fig. 1) is seen between the pulleys of the gill-rods of the two hemibranchiae of one gill („elastisches Band“ of RIESS, 1881, p. 527). In the other species, which are investigated, this ligament is not found, though in some fishes thin transverse fibres are visible. In reality this ligament is a thin transverse layer of elastic tissue connecting both hemibranchiae throughout the whole gill. In the microscopical sections the ligament is undulating. It is evident, as the examination by the binocular microscope shows, that in an animal, just after decapitation, this layer is stretched. It is obvious, that this apparatus plays a part in the maintaining of the normal position of the gill-filaments in quiet respiration.

Parallel to the length of the gill in some fishes an elastic longitudinal band was found (stainable by the method of WEIGERT for elastic tissue) between the two filament-rows. It runs along the arteria branchialis just where the afferent vessels for the filaments branch off from their principal vessel (plate XII fig. 1). In microscopical sections the band seems undulating by the alternating arrangement of these branches of the arteria branchialis. It is not possible to examine the real state of this mass of tissue in the just decapitated animal. On the level of every gill-rod elastic fibres branch off from this band of tissue, diverge along the wall of the afferent blood-vessel for the filament, which they enclose and can be followed as far as the perichondrium of the gill-rod. In this way perhaps this ligament can be of significance for the maintaining of the alternate arrangement of the filaments of the two hemibranchiae, which form a gill.

In the *Cyprinidae* and the *Salmonidae*, where the above mentioned ligament is absent, a strand of tissue is found nearer to the tip of the filament just at the end of the diaphragm, running parallel to the length of the gill (fig. 6). This strand of tissue, which probably has the same significance as the ligament described above, is connected with the tendons of the adductor muscles for the successive filaments of both hemibranchiae, as we will see afterwards. So in these animals there is a mechanism for the maintaining of the alternate arrangement of the filaments as well.

5. *The active part of the apparatus of movement of the gill-filaments.*

In the tissue between the two hemibranchiae of one gill small muscles are found, which, according to their origin and insertion, can act as adductor muscles for the filaments of both hemibranchiae. Agreeing with the conception of RIESS two types of adductor muscles can be distinguished in the material of these investigations. However neither RIESS nor I had the opportunity to investigate the sturgeon, which is described by DUVERNOY as representative of a third type of adductor muscles. A definite answer to the question of the existence of this third type cannot yet be given, though it seems probable, comparing the descriptions of DUVERNOY with the microscopical sections of the gills investigated by myself, that the adductor muscles of the sturgeon can be classified under the second type.

a. *The adductor muscles of the first type.*

Every muscle has its origin in the broadened basal extremities of two successive gill-rods of a hemibranchia. Near the origin, the muscle lies between these two gill-rods, then it crosses obliquely towards the other hemibranchia and it is inserted into the gill-rod of the alternating filament (plate XII fig. 2). In *Gobius auratus* and *Periophthalmus koelroeteri* Miss E. SCHÖTTLE describes a second origin of these muscles from the gill-bar. In the gills investigated by myself such an origin was not found. According to the length of the branchial diaphragm in this type of gills the length of the muscular part of every muscle is only one third or one fourth of the length of the filament. The long tendon of the muscle divides into two parts, enclosing the branch of the arteria branchialis for the filament, which runs along this side of the gill-rod. The two parts unite again to form one tendon, which is attached to the gill-rod along the whole of length.

Every filament has its own muscle; the successive muscles cross, but are entirely separated from each other.

By stimulating the muscle and observing the action through the binocular microscope, it is evident, that contraction of the muscle gives a hinge movement to the filament with regard to the gill-arch (fig. 5). The free endings of the filaments of both hemibranchiae of a gill approach each other, in the way, as RIESS

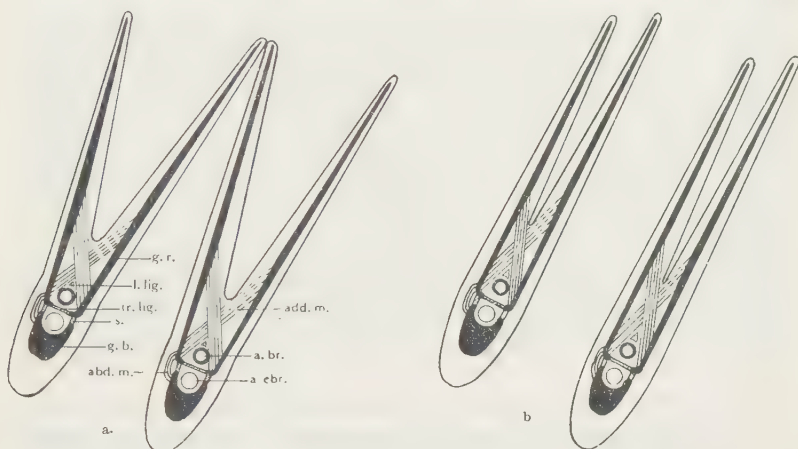


Fig. 5. A. Schematic diagram of a section through two successive gills with adductor muscles of the first type.

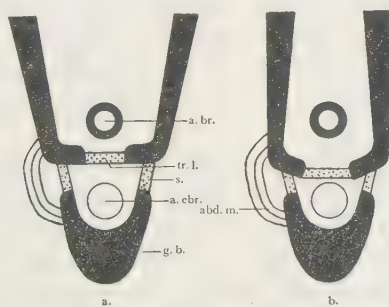


Fig. 5. B. Schematic diagram of the position of the gill-rods with regard to the gill-bar in higher magnification.

a. during quiet respiration; b. during the coughing movements.

g.b.: gill-bar; g.r.: gill-rod; s.: syndesmosis between gill-rod and gill-bar; tr.lig.: transverse ligament between the bases of the gill-rods; l.lig.: longitudinal ligament; a.br.: arteria branchialis; a.ebr.: arteria cpibranchialis; add.m.: adductor muscle; abd.m.: so-called abductor muscle.

described it (1881). The view of DUVERNOY (1839), that the action of these muscles should bend the free extremities of the filaments towards each other is not to be supported for this type of muscle.

Muscles of this type are found in: *Esox lucius*, *Umbra krameri*, *Cobitis barbatula*, *Ameiurus nebulosus*, *Gasterosteus aculeatus*, and *Perca fluviatilis*.

b. The adductor muscles of the second type.

Every muscle originates in the same way as the muscle of the first type from two successive gill-rods of one hemibranchia. The origin however does not lie at the base of the filament, but is shifted in the direction of the tip of the filament (plate XII fig. 3). The tendon crosses again to the alternate filament of the other hemibranchia and encloses the branch of the arteria branchialis for the filament, before it is inserted into the gill-rod.

The shifting of the origin of these muscles to the midst of the filaments goes parallel with the lengthening of the "diaphragme branchial", which reaches at least half-way down the filaments or even more.

The muscles of this type have given up their independance for a good deal, as the tendons of the successive muscles are united firmly by a ligament, running between the two hemibranchiae of the gill (fig. 6). This ligament forming the extreme end of the "diaphragme branchial" is covered with the mucous membrane of the gills and connects the tendons of the crossing muscles just there, where muscle and tendon merge into each other. This is the ligament mentioned on p. 253 by which the alternate arrangement of the filaments of the two hemibranchiae in these gills is maintained.

By stimulating these muscles and observing the action through a binocular microscope it is evident, that the filaments do not pivot in regard of the visceral bars, as we have seen in the gills with muscles of the first type, but that the slightly curved endings of the filaments straighten (fig. 6), which causes an adduction of the filament-tips of both hemibranchiae of a gill as well. This action is in agreement with the view of DUVERNOY (1839), but it is diametrically opposed to the conception of RIESS (1881), according to whom the muscles of this type act as abductors for the tips of the filaments notwithstanding the fact, that he calls them adductor muscles.

Muscles of this type are found in: *Tinca tinca*, *Leuciscus rutilus*, *Cyprinus carpio*, *Phoxinus laevis*, *Rhodeus amarus*, *Salmo irideus*.

c. The so-called abductor muscles.

In most of the species investigated here a thin sheet of muscle fibres is present at the base of the oral hemibranchia of every gill. No separate muscles are to be distinguished in this sheet. The muscle fibres originate in the oral side of the gill-bar (plate XII fig. 2 and textfig. 5). Where as in *Tinca tinca*, *Leuciscus rutilus*, *Umbra krameri*, *Cobitis barbatula* the gill-bar supports the long gill-rakers of a sieve-apparatus for the gill, muscle-fibres now and then originate from these. The muscle-fibres are inserted into the basal extremities of the gill-rods either immediately on the oral side of them (*Esox lucius*) or they penetrate into the space between gill-rod and gill-bar and are attached into the gill-rod on this side. In the caudal hemibranchia of every gill this sheet of muscle-fibres is absent. In *Rhodeus amarus* and *Gasterosteus aculeatus* we did not find these muscle-fibres at all. This sheet of muscle-fibres is described in literature as abductor muscles. The function of this muscle-sheet will be discussed in the next chapter.

6. The mechanism of movement of the gill-filaments.

In the introduction already is pointed out, that the communi-

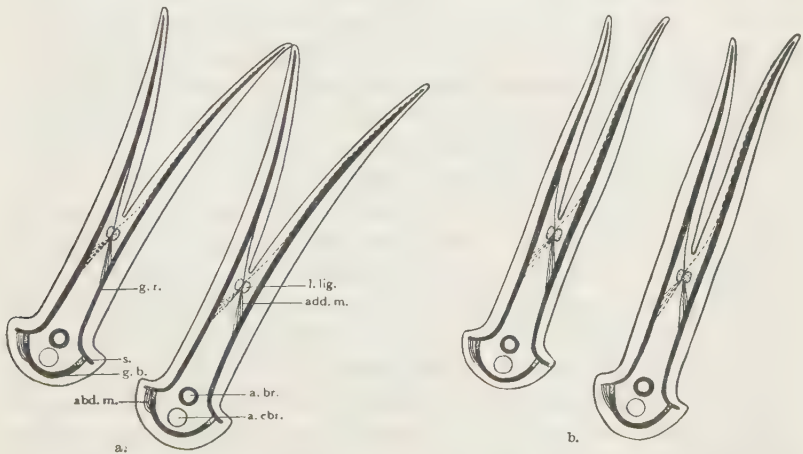


Fig. 6. Schematic diagram of a section through two successive gills with adductor muscles of the second type.

a. during quiet respiration; b. during the coughing movements.

Indications as in fig. 5.

cation between the inspiratory and the expiratory cavity is only kept up by the very narrow slits between the numerous lamellae, because the filament-tips of the posterior hemibranchia of each gill are in continuous contact with those of the anterior hemibranchia of the next gill. In this way the wide apertures between the gills are closed. In normal respiration fishes make coughing movements now and then, more frequently in water, in which small particles are suspended. During these coughing movements only for a short moment a wide communication exists between the inspiratory and the expiratory cavity, as the filaments, bridging the gill-slits, part from each other. Both hemibranchiae of a gill make an adduction movement. Mrs. HOFDIJK-ENKLAAR showed, that these adduction movements are to be called forth by mechanical stimulation of the mucous membrane in the neighbourhood of the filaments.

Afterwards I found, that the movements could be brought about easily by electrical stimulation and that it was possible by tetanical stimulation to fix the position of adduction as long as necessary to study it. Under normal circumstances the movement occurs so quickly, that a close examination is out of question.

Now it was evident, that the movements of adduction of the filament-tips in the representatives of both groups, distinguished after the two types of adductor muscles, occur in different ways. In *Esox*, as representative of the fishes with adductor muscles of the first type, the muscle moves the whole filament, which pivots in the junction between the gill-rod and the gill-bar round an axis passing through the broadened basal extremity of the gill-rod and running parallel with the gill-bar (fig. 5).

In *Tinca tinca* however, as a representative of the fishes with muscles of the second type, a movement between the base of the gill-rod and the gill-bar is not to be seen, but the curved endings of the filaments straighten (fig. 6). Differences in the structure of the filaments agree with these different ways of movement. The length of the branchial diaphragm, which reaches as far as the middle of the filament or farther in gills with muscles of the second type will prevent a hinge movement of the filaments. In stead of this the filaments in gills of this type are curved to the side of the inspiratory cavity and by straightening the free endings of them, the same effect of adduction of the two hemibranchiae forming a gill is obtained.

In both groups of fishes the opening of the gill-clefts during the

coughing movements occurs by an active adduction of the filament-tips. These movements are quite different from the passive movements mentioned in the first chapter and observed by Mrs. HOFDIJK-ENKLAAR on lifting a gill-cover. This opening of the gill-clefts was the result of an abnormal difference in pressure between inspiratory and expiratory cavity. During the coughing movements the contraction of the adductor muscles must be responsible for the opening of the gill-clefts.

It is more difficult to suppose, that the thin sheet of muscle fibres, found at the base of the oral hemibranchia of every gill in a number of species, should have an abducting function with regard to the filaments. In those gills, where the filaments pivot in the junctions between the gill-rods and the gill-bar, an abductor muscle could have sense. However the so-called abductor muscles are only present at the oral hemibranchia of every gill, moreover some species with adductor muscles of this type lack them. On the contrary these so-called abductor muscles are present in a number of fishes with adductor muscles of the second type, where an abducting function however is absolutely out of the question, for those gills with adductor muscles of the second type make no movements in the junction between filament and arch. The idea, which we have on the mechanism of fixation and of movement of the gill-filaments as the result of our experiments and of the anatomical data differs from the general view.

The hydrostatic pressure in the mouth-cavity exceeds that in the gill-cavity in every phase of breathing, i.e. during expiration as well as during inspiration (v. DAM 1938, p. 82). In this way a continuous current of water along the lamellae is guaranteed. This surplus of pressure in the mouth-cavity tends to adduct the filament-tips of both hemibranchiae of a gill. However the filament-tips of the posterior hemibranchia of every gill keep a close contact with those of the anterior row of the next arch in every phase of breathing. The old view, that in quiet breathing the position of the two filament-rows of a gill should be the result of a constant activity of the "abductor muscles" is wrong, as in the fishes with adductor muscles of the second type the muscle sheet concerned cannot act in this way. Every gill is constructed in such a way, that the filaments of both hemibranchiae diverge in both phases of normal respiration. The layer of elastic fibres (plate XII fig. 1), which is found in a number of species between the basal parts of the gill-rods of the two filament-rows belonging to

one gill and which is stretched in the just decapitated animal, must be considered as contributing to these powers. The elliptical form of the transverse section of the gill-rod and the longitudinal ridges increasing the greatest dimension of this section (described on p. 276) are structures, which help to prevent the filaments from being bent by the power of the current of water running from the mouth into the gill-cavity. By lifting the gill-cover (experiments of Mrs. HOFDIJK-ENKLAAR, see p. 269) the hydrostatic pressure in the gill-cavity decreases. Now that the difference in pressure between the inspiratory cavity and the expiratory cavity increases, the filament-tips, bridging the gill-clefts, lose their contact and the clefts open. This adduction of the filament-tips of the two rows of one gill occurs in a passive way.

Now in the coughing movements, which appear more frequently, as the animals breath in water laden with small suspending particles, we see an opening of the gill-clefts as well.

The coughing in a backward direction is nothing but a powerful expiration. As the difference in pressure between mouth- and gill-cavity increases in such a vigorous expiration, the opening of the gill-clefts perhaps could occur in a passive way. It is obvious however, that the adduction of the filaments occurs more powerful and must be the result of muscle action. The adductor muscles now come into contraction during a short moment.

The coughing in a forward direction is not possible without the action of the adductor muscles, as the current of water is now driven from the expiratory into the inspiratory cavity. The difference in hydrostatic pressure, which evokes this current of water cannot have an adductive action with regard to the filaments. The adduction of the filaments is stronger in the coughing movements in forward direction than in backward direction.

As soon as the sudden contraction of the adductor muscles is ended, the filaments are brought back into their original position by elastic powers, which are evoked by the adduction movement of the filaments in the elastic ligament (fig. 5b), in the syndesmosis between gill-rod and gill-bar, and in the syndesmotomic connections between the moving gill-rod and his neighbouring ones.

It is not probable, that the thin muscle sheet at the base of the oral hemibranchia, which is only present in a number of fishes, acts as an abducting power to fix the filaments in their normal position. First of all this muscle sheet is only found at the base of one of the two hemibranchiae of a gill, in the second place it is not

probable, that it acts as an abducting power in gills with muscles of the second type (p. 280). It is better to find an explanation for the function in another direction. As the gills have a latero-caudal position in the living animal, a movement of the two adducted hemibranchiae of a gill in a caudal direction may close the following gill-cleft. Specially during the coughing movement in a forward direction, in which the current of water flows from the expiratory into the inspiratory cavity, the pressure of the water promotes this enclosure of the gill-clefts. It is possible, that the muscle-fibres of the sheet mentioned above may prevent this obstruction of the gill-clefts during the coughing movements, specially during those in forward direction. One can imagine, that the contraction of this muscle-sheet fixes the adducted hemibranchiae in their latero-caudal position. In this way the muscle-sheet is not an antagonist with regard to the adductor muscles, but acts simultaneously with them.

7. *Significance of the adduction movements.*

Several authors (RIESS 1881, WOSKOBOINIKOFF 1932, ELFRIEDE SCHÖTTLE 1932) ascribe to the adduction movements of the filament-rows of a gill a significance for the renewal of the water during breathing and for the propulsion of the blood through the vessels of the gill-filaments. ELFRIEDE SCHÖTTLE (1932, p. 10) even speaks of fanning movements ("fächernde Bewegungen") of the gill-filaments in the Gobiiformes, brought about by the alternate action of the adductor muscles and the so-called abductor muscles, through which the respiration water should be renewed. According to RIESS the blood of the arteria branchialis should be pushed by the heartpower only as far as the branches for the filaments. By a successive contraction and relaxation of the adductor muscles the afferent vessels for the filaments should be closed and opened alternately, which would cause the current of the blood through the filaments.

It is evident, that these authors start from the supposition, that the adductor muscles have a rhythmical action, in which contractions and relaxations follow each other continuously. This supposition is untenable. The fact, that the adduction movements of the filaments only appear now and then, is easy to establish. It is out of the question that the branches for the filaments of the branchial artery should be closed during the action of the adductor muscles. The afferent vessels of the filaments have such a

position with regard to the gill-rods, that an action of pressure is avoided.

RAUTHER discusses this question in the same way in his description of the *Syngnathidae* (1925, 1937).

The adduction movements of the filaments however are seen only in connection with coughing movements in a forward, as well as in a backward direction. Small particles of defilement, which may accumulate against the gills, are removed in this way. The adduction movements are of no importance for the circulation of the blood through the branchial vessels, nor for the renewal of the respiratory water; they are only of significance for the cleaning of the gills. The adductor muscles open the gill-clefts by adducting the two filament-rows of a gill, and the thin muscle sheet at the oral hemibranchia of a gill, the so-called abductor muscles, probably prevents the caudal movement of the adducted filament-rows, which should close up the branchial apertures. It is possible, that these so-called abductor muscles act exclusively during coughing in a forward direction.

SUMMARY

It is evident, that the wide apertures between the gills are closed in quiet respiration, during inspiration as well as during expiration, because the filament-tips of the posterior hemibranchia of each gill are in continuous contact with those of the anterior hemibranchia of the next gill. The communication between the inspiratory and the expiratory cavity is kept up by the narrow slits between the lamellae. The skeleton and the ligament apparatus of the filaments are constructed in such a way, that the normal position of abduction of both filament-rows of a gill is maintained in spite of the fact, that in both respiratory phases the pressure in the inspiratory cavity is higher than that in the expiratory cavity. The figures of gills of the *Teleostei*, which are found in different textbooks, show a wide aperture between the filament-tips of the hemibranchiae of two successive gills. In the living animal this wide communication between the inspiratory and the expiratory cavity does not exist in quiet respiration, but is only seen during the coughing movements. Small fragments of defilement, which are accumulated against the gills, are then removed and are spit out by the mouth (coughing in a forward direction) or appear from under the gill-cover with the respiratory water (coughing in backward direction). During coughing an adduction movement

is seen of the filament-tips of the two hemibranchiae of a gill by the contraction of the adductor muscles.

The adductor muscles do not act rhythmically. They play no part in the circulation of the blood through the branchial vessels (RIESS 1881, WOSKOBONIKOFF 1932), nor in the renewal of the respiratory water (ELFRIEDE SCHÖTTLE 1932), but they are of significance for the cleaning of the gills by means of the coughing movements.

During coughing the so-called abductor muscles probably prevent a caudal movement of the adducted filament-rows of a gill, by which the branchial apertures could be closed up. It is possible, that they act exclusively during coughing in a forward direction.

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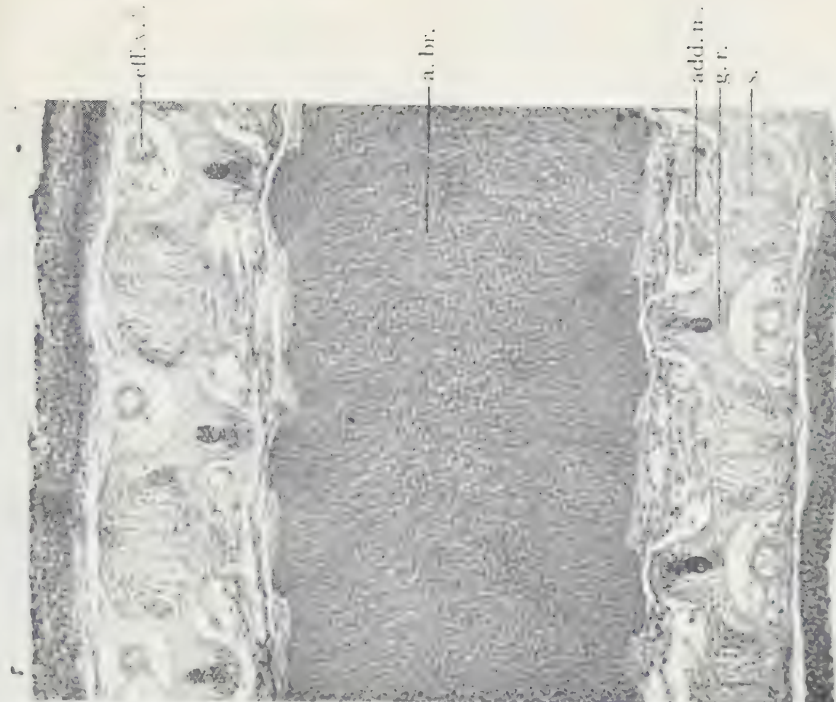


Fig. 2.

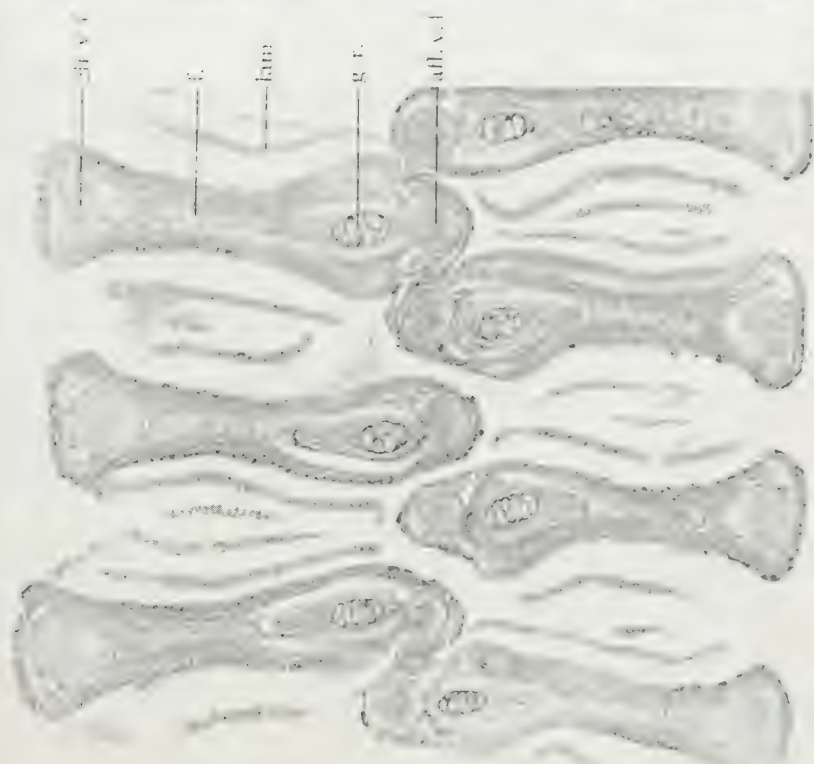


Fig. 1.

Fig. 1. Section of a gill of *Psoa borealis* perpendicular to the filaments. The alternate position of the adductor filaments is clearly to be seen. l., lamella; lam., lamina; l., efferent vessel of the filament; eff. v. l., efferent vessel of the filament; Fig. 2. Section of a gill of *Psoa borealis* going through the broadened basal extremities of the gill-rods; a. br., arterial branch bud; g. r., basal extremity of the gill-rod; s., syndesmosis between two gill-rods; eff. v. l., efferent vessel of the filament; add. m., adductor muscle section; add. m., adductor muscle section.

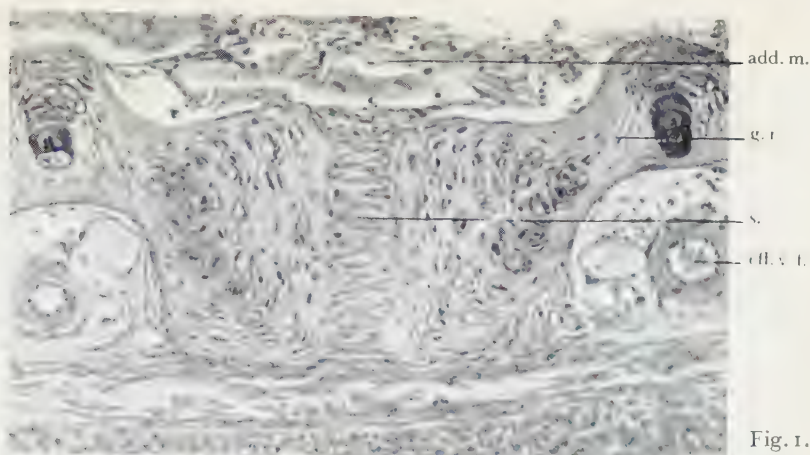


Fig. 1.

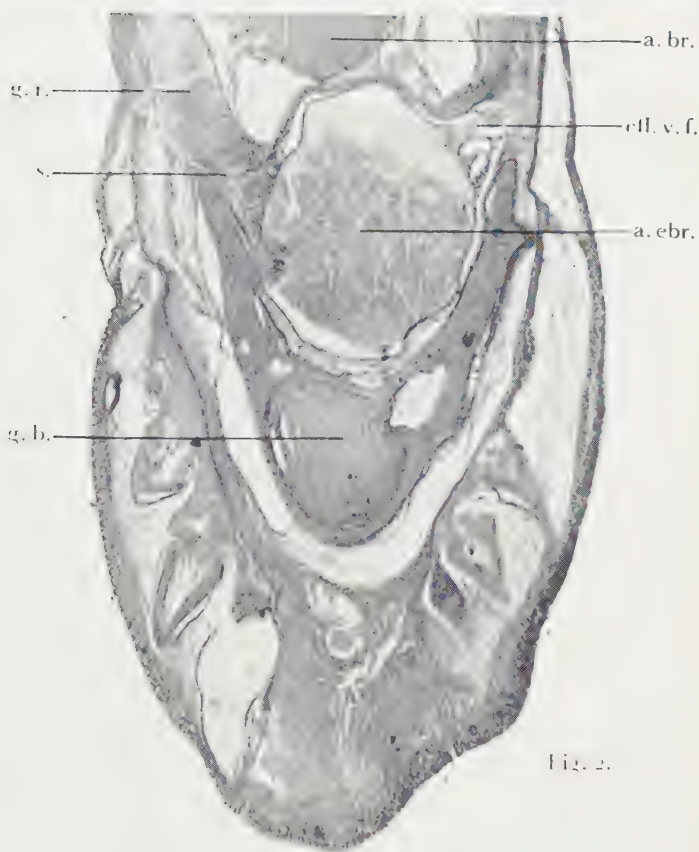


Fig. 2.

Fig. 1. Section through the basal extremities of the gill-rods in higher magnification. s.: syndesmosis between two gill-rods; eff.v.f.: efferent vessel of the filament; add.m.: adductor muscle sectioned near the origin.

Fig. 2. Section through the base of a gill of *Esox lucius*, showing on the left side the connection of the gill-rod and the gill-bar, on the right side the junction of the efferent vessel of the filament with the arteria epibranchialis. g.b.: gill-bar; g.r.: gill-rod; s.: syndesmosis; a.br.: arteria branchialis; a.ebr.: arteria epibranchialis; eff.v.f.: efferent vessel of the filament.

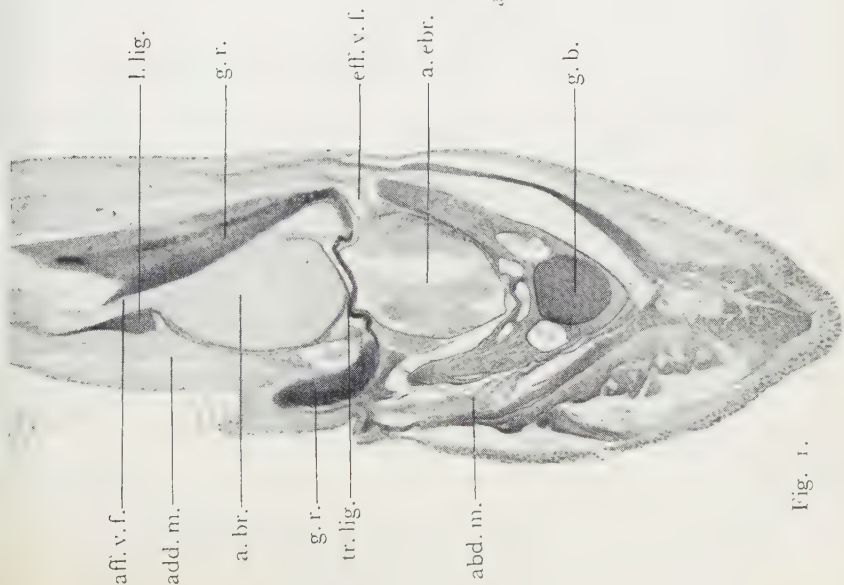


Fig. 1.

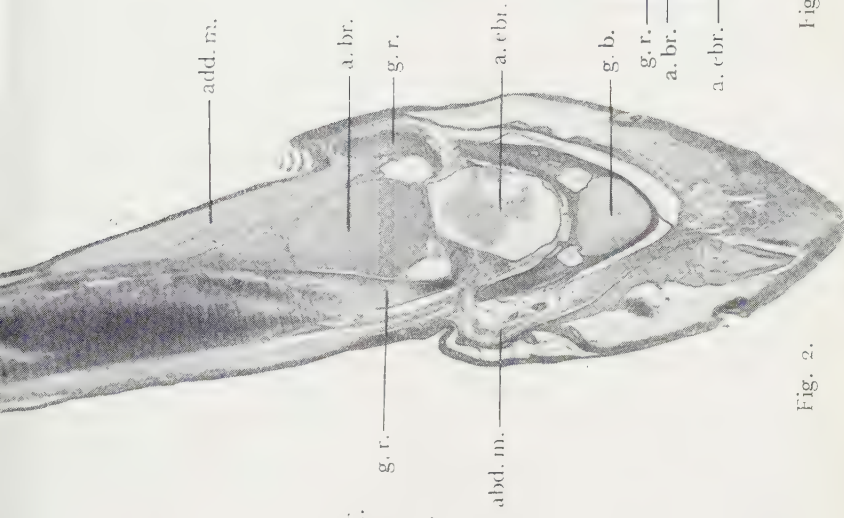


Fig. 2.

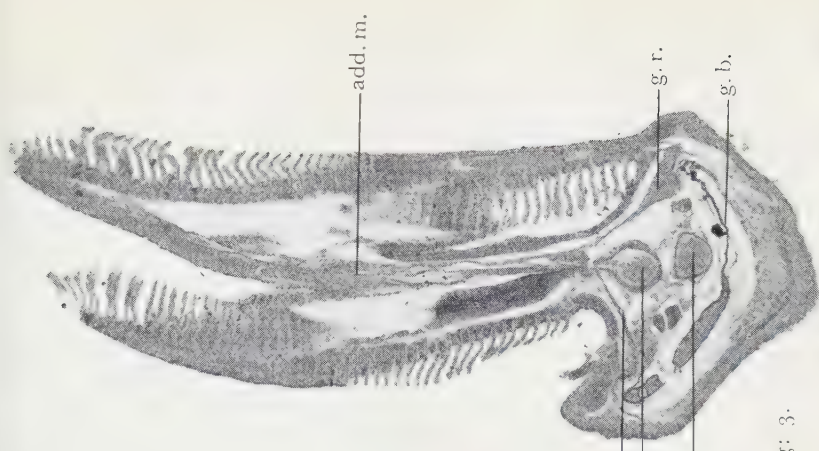


Fig. 3.

Fig. 1. Section through a gill of *Esox lucius*, stained after the method of Weigert for elastic tissue. tr.lig.: transverse ligament between the bases of the gill-rods consisting of elastic tissue; l.lig.: longitudinal ligament consisting of elastic tissue; g.b.: gill-bar; g.r.: gill-rod; a.br.: arteria branchialis; a.ebr.: arteria epibranchialis; aff.v.f.: afferent vessel of the filament; eff.v.f.: efferent vessel of the filament; add.m.: adductor muscle; abd.m.: so-called abductor muscle, Fig. 2. Section through a gill of *Esox lucius* parallel to the filaments. g.b.: gill-bar; g.r.: gill-rod; add.m.: adductor muscle; abd.m.: so-called abductor muscle; a.br.: arteria branchialis; a.ebr.: arteria epibranchialis. Fig. 3. Section through a gill of *Tinca tinca* parallel to the filaments. g.b.: gill-bar; g.r.: gill-rod; a.br.: arteria branchialis; a.ebr.: arteria epibranchialis; add.m.: adductor muscle.

BRYOZOA OF THE NETHERLANDS

BY

A. W. LACOURT.

LEIDEN

On the occurrence and the distribution of the Bryozoa in the Netherlands only little has been published up to now; this contrary to the surrounding countries of Western Europe where their occurrence is better known.

In 1826 BENNET EN VAN OLIVIER published a summary of the Bryozoa then known. MAITLAND (1851) listed a number of them, and once more in 1897. VIGELIUS (1884) enumerated Bryozoa collected in the Oosterschelde. VAN DER SLEEN (1929) dealt with the marine Bryozoa. VAN BENTHEM JUTTING (1922) and VORSTMAN (1936) treated with the Bryozoa of the former Zuiderzee.

I found also much information on the distribution of the Bryozoa in the lists of the investigations of the late DR. ROMIJN and of the "Rijksinstituut voor biologisch Visscherij-onderzoek" (the lists of the "Meerval").

The material put at my disposal belongs to the collections of the "Rijksmuseum van Natuurlijke Historie" at Leiden (ML), the "Zoologisch Museum" at Amsterdam (MA), the zoological laboratories at Amsterdam, Leiden and Utrecht, the "Zoologisch Station" at den Helder, the "Rijksinstituut voor biologisch Visscherij-Onderzoek," preserved in the "Hugo de Vries Laboratorium" at Abcoude (RVO), the "Comité ter bestudeering van de Nederlandsche mariene Fauna" at 's Gravenhage (Com) and my own (coll. A). I express my thanks to all, also to a number of private persons, who furnished me material and information. Though the material is not very extensive, together with literature and information it gives an impression of the occurrence of the Bryozoa in the Netherlands. Further investigations must be made to get a more detailed knowledge, for from many parts of the country nothing or but little is known. Up to now 78 species have been found, one of them and some varieties I propose to be new.

According to VAN DEINSE (1931 Proefschrift p. 154) and VAN REGTEREN ALTENA (1937 Proefschrift p. 3), the coast is divided into three parts: part I is the region from the Belgian frontier to

Hook of Holland, part II lies between Hook of Holland and den Helder, part III from den Helder to the German frontier.

The following list could be compiled:

A. MARINE BRYOZOA.

Ordo Cyclostomata.

Crisidia cornuta (L.).

HINCKS 1880, p. 419, pl. 56: 1-4; MARCUS 1940, p. 37, fig. 18.

I: fort den Haak on Walcheren, on seaweed and corallines, (MAITLAND 1851).

II: Noordwijk, Scheveningen; both on cork (ML).

III: Terschelling, on cork (ML).

Crisia eburnea (L.).

HINCKS 1880, p. 420, textfig. 21, pl. 56: 5-6.

I: Oosterschelde (VIGELIUS 1883, ML).

II: Scheveningen (ML, Com), Katwijk (coll. A), Zandvoort-IJmuiden (ML), den Helder (VAN DEINSE in litt.).

III: Terschelling (ML).

On *Flustra foliacea* (L.), hydroids, cork.

Crisia aculeata Hassall.

MARCUS 1940, p. 43, fig. 22.

II: Noordwijk (ML), on cork.

Crisia denticulata (Lamarck).

HINCKS 1880, p. 422, pl. 56: 7-9.

I: Westkapelle, on *Flustra foliacea* (L.). (MAITLAND 1851).

Tubulipora phalangea Couch.

MARCUS 1940, p. 62, fig. 32.

II: Scheveningen, on *Saccorhiza bulbosa* (Huds.), (ML); Noordwijk, three young colonies (?) on cork (ML).

Up to now not found in the Northsea.

Tubulipora liliacea (Pallas).

Idmonea serpens L., HINCKS 1880, p. 453, pl. 60: 2, pl. 61: 2-3;

Tubulipora liliacea (Pallas), BORG 1930, p. 45, fig. 23-24.

I: Westkapelle (MAITLAND 1851).

II: Scheveningen (Com), Katwijk (ML), Noordwijk (ML), Castricum (ML), den Helder (MA).

On seaweed, on a limb of *Lithodes maja* Leach, on cork.

Berenicea patina (Lamarck).*Diastopora patina* Lamarck, HINCKS 1880, p. 458, pl. 66: 1-6;*Berenicea patina* (Lamarck), BORG 1930, p. 48, fig. 26-27.

II: Scheveningen, Wassenaar, Katwijk, Noordwijk, den Helder, (ML and MA). On cork.

Hornera lichenoides (L.).

HINCKS 1880, p. 468, pl. 67: 1-5.

III: Westgat near den Helder, (HOFFMANN in VAN DER SLEEN 1919).

Lichenopora hispida (Fleming).*Lichenopora (Disporella) hispida* (Fleming), BORG 1930, p. 51, fig. 32.

II: Scheveningen (ML), Noordwijk (Com). On cork.

Lichenopora verrucaria (Fabr.).

BORG 1930, p. 52, fig. 33.

II: Zandvoort-IJmuiden (ML), on hydroids; den Helder (MA), on *Lithodes maja* Leach.

Ordo Cheilostomata.

Aetea anguina (L.).

HINCKS 1880, p. 4, pl. 1: 4-5.

II: Scheveningen, one zoarium measuring 30 x 15 mm, on cork. (Com).

Aetea sica (Couch).*Aetea recta* Hincks, HINCKS 1880, p. 6, pl. 1: 6-7.

II: Scheveningen, one zoarium measuring 15 x 9 mm, on cork. (Com).

Scruparia chelata (L.).*Eucratea chelata* L., HINCKS 1880, p. 14, pl. 1: 3, pl. 2: 4-8, pl. 3: 9-11.

II: Scheveningen (ML), Katwijk (MAITLAND 1851 and ML), Noordwijk (ML).

III: Terschelling (ML).

On seaweed, cork, *Scrupocellaria scruposa* L. on cork, on *Campanularia verticillata* (L.), on *Polysiphonia*.*Eucratea loricata* (L.).*Gemellaria loricata* L., HINCKS 1880, p. 18, pl. 3: 1-4.

I: Domburg (MAITLAND 1851).

II: Scheveningen (ML), IJmuiden (MA) on trawler, den Helder (VAN DER SLEEN 1919, communicated by TESCH).

Membranipora (Membranipora) membranacea (L.).

HINCKS 1880, p. 140, pl. 18: 5-6.

I: Westenschouwen (VAN DER SLEEN 1919), de Beer (Com).

II: Katwijk (MAITLAND 1851 and coll. A), along the shore from IJmuiden to den Helder (VAN DER SLEEN 1919, BENNET EN VAN OLIVIER 1826, VAN DEINSE in litt.).

III: the island of Ameland (VAN DEINSE in litt.), on amber; Groningen (MAITLAND 1851, BORGHORST 1944).

On seaweed, stones, shells, hydroids, crabs. This species, however, may be confused with *Membranipora (Electra) crustulenta* (Pallas). It has also been found in brackish water: the former Haarlemmer meer, the former IJ and the former Zuiderzee respectively (BENNET EN VAN OLIVIER 1826, MAITLAND 1851, VAN BENTHEM JUTTING 1922).

Membranipora (Electra) pilosa (L.).

Flustra pilosa L., BENNET EN VAN OLIVIER 1826;

Membranipora pilosa (L.), HINCKS 1880, p. 137, pl. 23: 1-4; NORMAN 1894, p. 115, MAITLAND 1851 and 1897, VIGELIUS 1883, VAN DER SLEEN 1919, VAN BENTHEM JUTTING 1922, VORSTMAN 1936.

I: Oosterschelde, the seashore of the island of Walcheren, Brouwershavensche Gat, Ierseke, the seashore of the island of Schouwen, Lake of Rockanje †, de Beer.

II: along the whole shore in this part.

III: Oude Schild on the island of Texel, Terschelling, Scheurak in the Waddenzee, Groninger Wadden, on a pier near Westerland on Wieringen. (Material in ML and MA.)

On all kinds of substratum: seaweed, shells, hydroids, egg-capsules of *Nucella lapillus* (L.) and of Rajidae, crabs, worm-tubes, Bryozoa, cinder, cork, drift-wood, pilework.

This species is abundant and extremely variable: the zooecia may be short or elongated, strongly calcified or hyaline; the wall thickened or thin and sharp; when elongated, commonly narrow; when short, commonly small all over. Often the zooecia are deformed: distorted, cup- bowl- or urn-shaped. The length and the number of the marginal spines also vary greatly, they may be absent but also luxuriantly developed, forming a hairy tuft. Though connected by intermediate forms, several formae can be distinguished and it is useful to fix them by a name, as far as this has not been done. HINCKS (1880, p. 138) has given already a brief description of this subject, NORMAN (1894, p. 115) has paid much attention to it and I could observe many of the character-

istics they mention. I do not follow NORMAN, except in one case, for his varieties based on the form of the zoaria, are due to the kind of substratum on which they were growing; they have no real value.

The formae refer: 1st to the shape of the zooecia and 2nd to the mode of growth of the zoarium.

Forma *typica* Levinsen (1894, p. 54), (HINCKS 1880, pl. 23: 1): opesia surrounded by a number of small spines, proximally a large corneous spine, abundant;

forma *dentata* Ellis and Solander (1786), (HINCKS 1880, pl. 23: 2): stout zooecia with numerous marginal spines, which are short and pointed and bend over the opesia, without proximal spine, common;

forma *tridentata* Loppens (1906), (HINCKS 1880, pl. 23: 4): with only three spines round the opesia, abundant;

forma *quinquedentata*, forma nova; with five spines round the opesia, abundant;

forma *luxurians*, forma nova: with numerous, very long, corneous spines round the opesia, the proximal spine being multiplied, rare;

forma *tenuis* Norman (1894, p. 117, pl. VI: 2): delicate, with very thin wall and short spines, lustrous, common;

forma *callosa*, forma nova: small, strongly calcified, all spines usually slightly developed, common;

forma *laxa* Smitt (1868, p. 370): zoarium consisting of lines of zooecia, rare;

forma *erecta* Loppens (1906, p. 136): from the adherent zoarium bilaminar shoots originating from lateral superfluous buds arise, possibly by want of space on the substratum. The buds and the zooecia in the shoots are often deformed, rather common.

It seems possible to me that shoots which are torn off can live pelagically and grow on. I have found zoaria consisting of a number of shoots, growing in the mode of forma *erecta*, which have the form of a ball and not or no longer were attached on a substratum; these zoaria were found in fresh condition with living polypides and apparently had lived in a floating state;

forma *verticillata* Ellis and Solander (1786, p. 15, Tab. 4): this forma has reference to the mode of arrangement of the zooecia, viz., in transverse rows which arrangement may occur also in the formae *tridentata*, *quinquedentata* and *erecta*. The spines are like those in the forma *typica*, common.

Membranipora (Electra) hastingsae Marcus.

MARCUS 1940, p. 118, fig. 62.

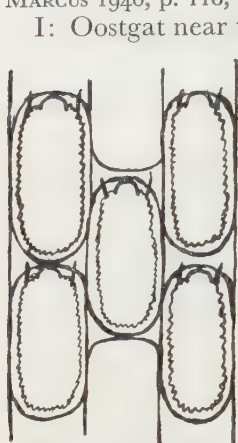


Fig. 1.

M. (Electra) hastingsae
Marcus.

Deviating zooecia out
of brackish water.

this species from its allies, specially from *Membranipora (Electra) crustulenta* (Pallas).

Membranipora (Electra) crustulenta (Pallas).

BORG 1930, p. 64, fig. 54 and 55; do. 1931, p. 27, pl. 3: fig. 4-5.

Eschara lapidescens, BASTER 1759 and 1762;

Millepora crustulenta Pallas, BENNET EN VAN OLIVIER 1826;

Flustra crustulenta (Pallas), MAITLAND 1851;

Hoornwiersoort (Eschara of Flustra), VAN BEMMELEN 1874;

Membranipora crustulenta (Pallas), LORIÉ 1896;

Eschara lapidescens Baster, MAITLAND 1897 and 1898;

Membranipora membranacea Linné, ROMIJN 1907-1925 (in Archives);

Palingbrood (Brachiopora crustulenta), JONKER 1912;

Eschara lapidescens Baster, BLAAUW 1917;

Membranipora crustulenta (Pallas), REDEKE 1918-1923 (in Archives);

Membranipora membranacea L. var. *erecta* Loppens, VAN DER SLEEN 1919; VAN BENTHEM JUTTING 1922;

Conopeum lacroixii Audouin, VAN KAMPEN 1925;

Membranipora crustulenta (Pallas), VAN MASTENBROEK 1927;

Membranipora membranacea L. var. *erecta* Loppens, OTTO EN WIELINGA 1933;

Membranipora crustulenta (Pallas), VORSTMAN 1936, DE VOS 1939;

Membranipora (Electra) crustulenta (Pallas) var. *fossaria* (HINCKS), VAN BENTHEM JUTTING 1946.

I: many localities in the streams, in Zeeuwsch Vlaanderen and on the islands of the province of Zeeland; Lake of Rockanje †;

II: "provincie Zuid-Holland", preparation of MAITLAND without date or locality; in the province of Noord Holland: Noordzeekanaal and many localities north of that canal;

III: brackish water round Harlingen in the province of Friesland and in the province of Groningen, some localities on the island of Texel, Westerland on Wieringen.

In the former Zuiderzee; in the adjacent brackish pool near Blankenham and in the Anne Ruardipool in the province of Overijssel. (Material in ML and MA).

On all kinds of substratum: stones, walls, lock-gates, campshot, waterplants. The species may live under different conditions: Cl 1287-4652 mg/l (ROMIJN in archives), 770-3040 mg/l (DE Vos 1939), in the province of Noord Holland, 1575-2425 mg/l (DE Vos 1939) in the province of Overijssel, 17644 mg/l (VAN BENTHEM JUTTING 1922) in the former Zuiderzee. So the limits lie, as far as known, between 770 and 17644 mg/l. - H_2CO_3 0,227-0,354 o/oo (ROMIJN in archives). - pH 7-8,5.

This species forms extensive zoaria which may cover large areas (VAN BENTHEM JUTTING 1922, photographs by VAN DER SLEEN). LORIÉ (1896) and BLAAUW (1917) have made investigations about incrustations and calcifications of this species in the Lake of Rockanje; the former, moreover, has examined a great number of brackish pools in the province of Zeeland on the occurrence of this species. VAN BENTHEM JUTTING (1946) has reported that this species "... rapidly took advantage of the new surroundings ..." during the inundation of the island of Walcheren, October 1944-October 1945 and that "... these minute animals within the course of one year can accumulate to mighty colonies of 200 mm and more in length ...".

There has been much confusion about this species until BORG (1931) has given us a clear explanation, dividing the species into several geographical varieties. According to his article, the material found in the brackish water of Holland belongs to the variety *fossaria* Hincks. Now it is remarkable that in one zoarium, found in the estuary of the river Schelde, the zooecia show much resemblance to the variety *arctica* Borg: they are much more calcified, they show a well-developed protuberance and chitinous spine; they much agree with plate I fig. 5 by BORG (1931); the zooecia are broader than those of the variety *fossaria*, the opercula

are more calcified, shorter and broader. This is a specimen from seawater, while in Holland this species usually has been found in brackish water!

Another specimen, from Enkhuizerzand in the entrance of the former Zuiderzee, also shows much calcification with well-developed proximal spine and broad operculum.

Membranipora (Conopeum) reticulum (L.).

Membranipora lacroixii Audouin, HINCKS 1880, p. 129, pl. 17: 5-8.

I: on the island of Walcheren: Domburg, Veere; on the island of Schouwen: Zierikzee, Burghsluis, Bruinisse; Oosterschelde, Veersche Gat, Brouwershavensche Gat, Oostgat. Lake of Rockanje †.

II: Kijkduin, Wassenaar, Katwijk, Noordzeekanaal: on pile-work of the Velsen ferry-boat, den Helder.

III: Scheurrak and Westmeep in the Waddenzee, Eierlandsche Gat, Vliestroom, Westerland on Wieringen, Noordpolderzijk in the lock. (Material in ML and MA).

On shells, *Balanus*, crab, cork, drift-wood, stone.

This species has often been found together with *Membranipora (Electra) crustulenta* (Pallas) in the province of Zeeland and in the Noordzeekanaal; it also has been found in the famous Lake of Rockanje but neither LORIE (1896) nor BLAAUW (1917) have seen this species among the material that they found in the lake.

The species shows sometimes marginal spines and nearly always the two characteristic kenozooezia near each autozooecium which, however, may show differences in form and place; in one specimen, from den Helder, the kenozooezia are not only developed proximally but also laterally, all around the zooecium. Sometimes two kenozooezia are fused together into a big one, sometimes they are much enlarged and deformed.

Total regeneration often happens in this species; in one case I have seen some double-zooecia as a monstrosity. As an example of strong growth I mention a zoarium on an oyster-shell that has reached a thickness of 25 mm by means of repeated deposition in layers.

Membranipora (Acanthodesia) tenuis Desor.

MARCUS 1940, p. 126, fig. 66.

I: West Hinder (MA).

II: Scheveningen (ML), Katwijk (coll. A), Noordwijk (ML).

In this species too the zoaria sometimes grow in thick layers on shells.

Membranipora (Tegella) unicornis (Fleming).

HINCKS 1880, p. 154, pl. 20: 4.

I: Zeeland (MAITLAND 1851).

II: Katwijk (MAITLAND 1851).

Membranipora (Callopora) lineata (L.).

HINCKS 1880, p. 143, pl. 19: 3-6.

I: some localities on the island of Schouwen (ML).

II: Terheyde (coll. A), Scheveningen (Com), Katwijk (coll. A), Noordwijk (ML).

III: Terschelling (Com).

On shells, seaweed, cork, stones.

Membranipora (Amphiblestrum) flemingii Busk.

HINCKS 1880, p. 162, pl. 21: 1-3.

II: den Helder, on a limb of *Lithodes maja* Leach (MA), "Nederland", rare, (MAITLAND 1897).*Membranipora (Ramphonotus) minax* Busk.

HINCKS 1880, p. 169, pl. 22: 2; MARCUS 1940, p. 142, fig. 76.

II, Scheveningen (ML). On cork.

Flustra foliaceae (L.).

HINCKS 1880, p. 115, pl. 14: 10, pl. 16: 1. 1a. 1b.

I: Breskens (MA), on the seashore of the island of Walcheren (ML), Oosterschelde (VIGELIUS 1883), Renesse on the island of Schouwen (Com), de Beer (Com).

II: along nearly the whole beach in this part from Hoek van Holland to Petten (Com, VAN DER SLEEN 1919, ML).

III: Vlieland (BENNET EN VAN OLIVIER 1826), Terschelling (Com), Ameland (Zoologisch Laboratorium Leiden), Groningen (MAITLAND 1851 and BORGHORST 1944).

(In the former Zuiderzee, MAITLAND 1851.)

On shells, a piece of peat, cork. Height of the zoarium: to 22 cm. Sometimes in enormous numbers.

One zoarium bears a superficial layer; in this layer the zooecia are not typical, they represent an intermediate form between *Flustra foliacea* and *Membranipora*. This layer has probably originated from a regenerated zooecium that became an ancestrula.

Some zoaria show dark zones which have been caused by (traumatic?) fractures; in these zones the zooecia are deformed, strongly enlarged, broadened or lengthened, with a pointed distal part or polygonal; sometimes multilocular cysts grow out from

these damaged zooecia, originated possibly by budding which was caused by a traumatic stimulus but not led to the formation of fullgrown zooecia.

According to HINCKS (1880) the zoarium is variable in shape, divided into lobes chiefly in two manners: either into broad lobes for which form the name *palmata* Hincks (1880, p. 117, pl. 14: 10) must be used or into narrow strips for which form I propose the name *secta* forma nova.

Flustra securifrons (Pallas).

HINCKS 1880, p. 120, pl. 16: 3-3a.

I: near fort den Haak on the island of Walcheren (MAITLAND 1851), Renesse on the island of Schouwen (Com).

II: Wassenaar (coll. A), Katwijk (Zoologisch Laboratorium Leiden), Zandvoort and IJmuiden (VAN DER SLEEN 1919), den Helder (VAN DEINSE in litt.), "Nederland", common, (MAITLAND 1897).

III: Groninger Wadden (BORGHORST 1944).

Cellaria salicornis (Pallas).

Cellaria fistulosa (L.), HINCKS 1880, p. 106, pl. 13: 1-4.

II: Katwijk-Zandvoort (MAITLAND 1851).

Tricellaria ternata (Ellis and Solander).

Menipea ternata (Ellis and Solander), HINCKS 1880, p. 38, pl. 6: 1-4.

II: Katwijk (MAITLAND 1851). On hydroid.

Scrupocellaria scruposa (L.).

HINCKS 1880, p. 45, pl. 7: 8-10.

I: Oosterschelde (VIGELIUS 1883), Sas van Gent (ML), Goes (Zoologisch Laboratorium Leiden), Westkapelle (MAITLAND 1851), on the shore of the island of Schouwen (ML and coll. A).

II: along the whole beach in this part (MAITLAND 1851, VAN DER SLEEN 1919, ML, MA, Zoologisch Laboratorium Utrecht).

On seaweed, shells, *Hyas araneus* (L.), Tunicata sp., *Flustra foliacea* (L.), sponge, cork, stone.

Scrupocellaria scabra (Van Beneden).

HINCKS 1880, p. 48, pl. 6: 7-11.

"Nederland" (MAITLAND 1897).

Scrupocellaria reptans (L.).

HINCKS 1880, p. 52, pl. 7: 1-7.

II: Terheyde (coll. A), Scheveningen (ML and Com), Katwijk (coll. A), Noordwijk (ML), Petten (coll. A).

On seaweed, *Flustra foliacea* (L.), cork.

Bicellariella ciliata (L.).

Bicellaria ciliata (L.), HINCKS 1880, p. 68, pl. 8: 1-5.

I: Westkapelle (MAITLAND 1851), Vlissingen (MA), Schelde near Goes (Zoologisch Laboratorium Leiden), on the shore of the island of Schouwen (ML).

II: Scheveningen (Com), Katwijk (coll. A), Noordwijk (ML), den Helder-Nieuwediep (ML and MA), numerous.

On hydroids, *Flustra foliacea* (L.), sponge, seaweed, cork.

Bugula avicularia (L.).

HINCKS 1880, p. 75, pl. 10: 1-4.

I: harbour and lock at Veere, canal through the island of Walcheren, (both in Zoologisch Laboratorium Leiden).

II: Katwijk, (MAITLAND 1851), shore of the Netherlands (BENNET EN VAN OLIVIER 1826), Scheveningen (Com).

(In the former Zuiderzee HORST 1885.) On hydroids and lock-gates.

Bugula plumosa (Pallas).

HINCKS 1880, p. 84, pl. 12: 1-5.

I: fort den Haak on the island of Walcheren (MAITLAND 1851), Vlissingen (MA), in the river Oosterschelde near Goes (Zoologisch Laboratorium Leiden) and near Ierseke (ML), Kisters Inlaag near Zierikzee (Zoologisch Laboratorium Leiden).

II: den Helder (ML), shore of the Northsea (BENNET EN VAN OLIVIER 1826).

(In the former Zuiderzee, Zoologisch Laboratorium Leiden.)

On Tunicata, hydroids, stones. Height of the zoarium: up to 8,5 cm.

Bugula flabellata (Thompson).

HINCKS 1880, p. 80, pl. 11: 1-3.

II: Scheveningen (ML). On cork.

Dendrobeatia murrayana (Johnston).

HINCKS 1880, p. 92, pl. 14: 2-9.

II: Petten (coll. A).

Membraniporella nitida (Johnston).

HINCKS 1880, p. 200, pl. 27: 1-8.

I: Heertjes Inlaag near Serooskerke on the island of Schouwen (ML).

II: Scheveningen (Com), den Helder (MA).

"Nederland" (MAITLAND 1897). On seaweed and cork.

Aspidelectra melolontha (Busk).

HINCKS 1880, p. 202, pl. 27: 9-10.

I: Oosterschelde: perceel 416 (coll. A) and near Gorishoek (ML), Oostgat (MA); Westerschelde near Ellewoutsdijk (coll. A), between Westkapelle and Domburg (coll. A).

II: Scheveningen (ML).

On recent and fossil shells.

Cribrilina annulata (Fabr.).

HINCKS 1880, p. 193, pl. 25: 11-12.

"Nederland" (MAITLAND 1897).

Cribrilina punctata (Hassall).

HINCKS 1880, p. 190, pl. 16: 1-4, pl. 14: 3.

I: Schelde (Zoologisch Laboratorium Leiden).

On *Mya arenaria* (L.).

Hippothoa hyalina (L.).

HINCKS 1880, p. 271, pl. 18: 8-10.

I: on the shore of the island of Schouwen (ML).

II: Terheyde-Wassenaar (Com), Katwijk (coll. A), Noordwijk (ML).

III: Terschelling (ML).

"Nederland" (MAITLAND 1897).

On *Flustra foliacea* (L.), *Patella*, seaweed, cork.

Chorizopora brogniartii (Audouin).

HINCKS 1880, p. 224, pl. 32: 1-4.

II: Scheveningen (Com). On cork.

Escharella immersa (Fleming).

Mucronella peachii (Johnston), HINCKS 1880, p. 360, pl. 50: 1-5.

Escharella immersa (Fleming), MARCUS 1940, p. 226, fig. 114.

II: Zandvoort (coll. A). On cork.

Peristomella coccinea (Abildgaard).

HINCKS 1880, p. 371, pl. 34: 1-6.

II: Scheveningen (Com), Noordwijk (ML). On cork.

Schizoporella unicornis (Johnston).

HINCKS 1880, p. 238, pl. 35: 1-5; MARCUS 1940, p. 237, fig. 121.

II: Scheveningen (Com). On *Patella vulgata* (L.).

Schizomavella linearis (Hassall).

HINCKS 1880, p. 247, pl. 38: 5-10, pl. 24: 1; MARCUS 1940, p. 244, fig. 125.

I: Oosterschelde: perceel 311 (coll. A).

II: Katwijk (coll. A). On shells.

Escharina spinifera (Johnston).

HINCKS 1880, p. 241, pl. 35: 6-8.

II: den Helder, Noorderhoofd (VAN DER SLEEN 1919). On stones.

Hippodiplosia pertusa (Esper).

HINCKS 1880, p. 305, pl. 43: 4-5, MARCUS 1940, p. 251, fig. 129.

I: Heertjes Inlaag near Serooskerke on the island of Schouwen.

Cryptosula pallasiana (Moll).

HINCKS 1880, p. 297, pl. 33: 1-3.

I: Oosterschelde (ML), on the island of Walcheren: Middelburg (MA), in the lock at Veere (VAN KAMPEN 1925, Zoologisch Laboratorium Leiden), on the island of Schouwen: Scharendijke (ML), Zierikzee (ML) and Serooskerke-Leinshoofd (ML), on stones of the dike. Also on oyster-tiles, *Ostrea* and *Balanus*.II: Scheveningen (Com), on cork; den Helder (VAN DER SLEEN 1919), on stones of the dike and on *Balanus* from the piers near the dam (MA).

Two monstrosities have come under my eyes: giant-zooecia, 3-5 times as large as normally with the orificium in the middle and two zooecia which were grown together, the orificia being fused.

Microporella ciliata (Pallas).

HINCKS 1880, p. 206, pl. 28: 1-8.

I: Oosterschelde (VIGELIUS 1884, ML), Flauwers Inlaag near Kerkwerpe on the island of Schouwen (ML).

II: Scheveningen (ML, Com), Katwijk (coll. A), Noordwijk (ML).

On shells, *Flustra foliacea* (L.), seaweed, stones, cork.*Smittina reticulata* (J. Macgillivray).

HINCKS 1880, p. 346, pl. 48: 1-5.

II: den Helder-Noorderhoofd (VAN DER SLEEN 1919). On stones.

"Nederland" (MAITLAND 1897).

Discopora arctica (M. Sars).*Mucronella pavonella* (Alder), HINCKS 1880, p. 376, pl. 39: 8-10.*Discopora arctica* (M. Sars), MARCUS 1940, p. 284, fig. 146.II: den Helder (MA), On a limb of *Lithodes maja* Leach.

Cellepora armata Hincks.

HINCKS 1880, p. 410, pl. 54: 10-13.

II: Terheyde (Com), Scheveningen (ML), Katwijk (coll. A), Zandvoort-IJmuiden (ML).

On hydroids, stones, cork.

Cellepora pumicosa (L.).

HINCKS 1880, p. 398, pl. 54: 1-3.

II: Scheveningen (Com), Katwijk (MAITLAND 1851), Noordwijk (ML).

On hydroids, cork, stones.

III: on the island of Ameland: the eastern part "het Oerd" (coll. A) a zoarium measuring: length 42 mm, breadth 38 mm, height 19 mm. This zoarium was growing on tubes of *Serpula*. Two more zoaria which were growing on tubes of *Serpula*, are known to me, viz., one from Horn Reef Grounds (MA), measurements: length 50 mm, breadth 40 mm, height 25 mm and one from Berry Head at Bringham (ML) resulting from repeated deposition in layers, five zones of growth distinctly visible.

Siniopelta costazii (Audouin).

HINCKS 1880, p. 411, pl. 55: 11-14.

I: Heertjes Inlaag near Serooskerke on the island of Schouwen (ML).

II: Scheveningen (Com), Katwijk (coll. A), Noordwijk (ML).

On *Patella*, seaweed, cork.

Ordo Ctenostomata.

Alcyonidium gelatinosum (L.).

Plate XIV A; Fig. 3A; Fig. 5A.

Halodactylus diaphanus Lamouroux, MAITLAND 1851 and 1897;*Alcyonidium gelatinosum* (L.), BENNET EN VAN OLIVIER 1826, VIGELIUS 1884, VAN DER SLEEN 1919, VAN BENTHEM JUTTING 1922, MARCUS 1940, p. 301, fig. 155.

I: along the shore of the islands of Walcheren and of Schouwen, Oosterschelde.

II: along the whole shore in this part.

(Material in ML, MA, zoological Laboratories at Leiden and Amsterdam.)

III: Westmeep (Zool. Station den Helder), Blauwe Slenk and Inschot in the Waddenzee (both in Com), Terschelling (Com).

Sometimes in enormous numbers. Specially found on *Buccinum*

undatum (L.), *Natica catena* (Da Costa), and the tube of *Mya arenaria* L., also on other shells, stones and drift-wood.

A certain number of zoaria shows a brownish-violet colour. It is not possible to me to explain this colour; microscopical examination shows the presence of capsulated organisms in the septa between the zooecia. The colour is brown or green. These organisms are not capsulated "brown bodies", for a structure is distinguishable. They may be parasitic Protozoa or Algae. I add a photo-micrograph (pl. XIII fig. 1) hoping that some zoologist will be able to identify them. The material is at his disposal.

Alcyonidium polyoum (Hassall).

Alcyonidium mytili (Dalyell), VAN KAMPEN 1925;

Alcyonidium polyoum (Hassall), MARCUS 1940, p. 302, fig. 156.

I: Heertjes Inlaag near Serooskerke (ML), Ierseke (Zoologisch Laboratorium Leiden), Vlissingen-harbour (VAN KAMPEN 1925).

II: Kijkduin (ML), Amsteldiep near van Ewijcksluis at Amsterdam (MA), den Helder-Nieuwediep (MA).

III: Scheurrak (MA), Westmeep (ML) and Wierbalg (MA) in the Waddenzee, Vlietstroom (ML).

(In the former Zuiderzee, VORSTMAN 1936.) On *Carcinus moenas* L., shells, seaweed, stones.

Alcyonidium hirsutum (Fleming).

Plate XIV B; Fig. 3B; Fig. 5B.

MARCUS 1940, p. 304, fig. 157.

I: Schouwen (MAITLAND 1851), Renesse (Com), Domburg (MAITLAND 1851).

II: Scheveningen (MAITLAND 1851), Katwijk and Zandvoort (VAN DER SLEEN 1919), IJmuiden and den Helder (MA).

III: Texelstroom (Zool. Station den Helder), Terschelling (MA). (In the former Zuiderzee, HORST 1885.) On shells, seaweed, pebble.

All zoaria belong to the erect form. One zoarium from the Northsea reaches a height of 38 cm.

Alcyonidium parasiticum (Fleming).

MARCUS 1940, p. 304, fig. 158.

I: Serooskerke (ML), Zeeland? (probably already mentioned by BASTER 1762 under a non-scientific name and not recognized by him as a Bryozoan).

II: from Hoek van Holland to Zandvoort (VAN DER SLEEN

1919), Scheveningen (MAITLAND 1851), "Nederland" (MAITLAND 1897), dredged off Scheveningen (ML).

III: Delfzijl (MA).

On hydroids, seaweed, Bryozoa.

Alcyonidium mamillatum Alder.

MARCUS 1940, p. 306, fig. 159.

I: Brouwershavensche Gat (ML), Anna Jacoba on St. Filips-land (ML).

II: dredged off Zandvoort, one mile out of shore (ML).

On crabs.

Flustrella hispida (Fabr.).

HINCKS 1880, p. 506, pl. 72: 1-5; MARCUS 1940, p. 308, fig. 161.

I: along the shore of the island of Schouwen (ML).

II: Scheveningen (ML), "Nederland" (MA).

On indigenous and exotic seaweed.

Vesicularia spinosa (L.).

HINCKS 1880, p. 513, pl. 73: 3-7.

I: Westkapelle (MAITLAND 1851), the island of Walcheren (ML), Haamstede on the island of Schouwen (ML).

II: Katwijk (BENNET EN VAN OLIVIER 1826, MAITLAND 1851), off Scheveningen (ML).

"Nederland" (MAITLAND 1897).

Amathia lendigera (L.).

HINCKS 1880, p. 516, pl. 74: 7-10.

II: Scheveningen (MA, MAITLAND 1851).

On seaweed.

Bowerbankia imbricata (J. Adams).

BORG 1930, p. 101, fig. 127; MARCUS 1940, p. 313, fig. 163.

I: Ierseke (ML), Oosterschelde (ML, VIGELIUS 1884), Bergsche Bank (MA), in the lock at Veere on the island of Walcheren (Zoologisch Laboratorium Leiden), Veersche Gat (MAITLAND 1851).

II: Helder-Nieuwediep (ML, VORSTMAN 1921).

On hydroids and seaweed.

Bowerbankia caudata (Hincks).

HINCKS 1880, p. 521, pl. 75: 7-8; BORG 1930, p. 101, fig. 128-129.

I: Veere on the island of Walcheren (Zoologisch Laboratorium Leiden).

(In the former Wieringermeer, VORSTMAN 1936.)

Valkeria uva (L.).

HINCKS 1880, p. 551, pl. 75: 1-5; MARCUS 1940, p. 318, fig. 166.

I: Ierseke (ML), Zeeland (BENNET EN VAN OLIVIER 1826, MAITLAND 1851).

II: den Helder (MA), Westgat near den Helder (VAN DER SLEEN 1919).

(In the former Zuiderzee, VAN BENTHEM JUTTING 1922.)

On *Flustra foliacea* (L.), hydroids, crabs, seaweed.

Farrella repens (Farre).

HINCKS 1880, p. 529, pl. 78: 5-6; BORG 1930, p. 103, fig. 134-135.

I: Oosterschelde (VIGELIUS 1884, ML), Veersche Gat (MAITLAND 1851), Brouwershavensche Gat (ML).

II: Kijkduin (ML), off Zandvoort one mile out of shore (ML), den Helder (ML, Zoologisch Laboratorium Leiden), along the whole shore (VAN DER SLEEN 1919).

III: Harlingen-harbour (?) (OTTO EN WIELINGA 1933).

(In the former Zuiderzee, MA, and in the former Wieringermeer ML.)

On seaweed, crabs, hydroids, stones.

* *Anguinella palmata* Van Beneden.

HINCKS 1880, p. 539, fig. 30; pl. 77: 5-5a.

II: den Helder-harbour (VAN DER SLEEN 1919).

III: Texelstroom (VAN BENTHEM JUTTING 1922).

(In the former Zuiderzee, VAN BENTHEM JUTTING 1922.)

Victorella pavida S. Kent.

HINCKS 1880, p. 561, pl. 79: 4-7; BORG 1930, p. 106, fig. 141-143.

II: Amsterdam: harbour and canals (ML, MA, Zoologisch Laboratorium Amsterdam), floating beam in the Zaan (MA), den Helder (ML). Schellingwouderbroek in the province of Noord Holland (DE VOS 1939).

On *Mytilus edulis* L., *Congerina cochleata* (Nyst), *Balanus improvisus* L., hydroids, seaweed, wood; in brackish water.

Paludicella articulata (Ehrenberg).

BORG 1930, p. 107, fig. 144-146.

Paludicella ehrenbergi Van Beneden, DE VRIES 1887, BLAAUW 1912, ROMIJN EN WIBAUT-ISEBREE MOENS 1920, VAN DER SLEEN 1920.

Part Z I: (see for these parts under Phylactolaemata): Langweerder Wielen, Foxholstermeer.

Part Z II: Drentsche A near de Punt, an old branch of the Beilerstroom near Lheebroek.

Part Z III: Sonsbeek: immediately above the waterfall and in a pond.

Part Z IV: in the river Vecht, in a pond near the Plantagehuis at Vreeland, in the Lake of Abcoude.

Part Z V: in the Ringvaart of the Haarlemmermeerpolder, Drecht near Leimuiden, Kagerplassen, Brasemermeer near Roe-lofarendsveen, Heimanswetering at Woubrugge, Zijl near Leiden, in the river Oude Rijn: Bodegraven, Zwammerdam, Alphen near Gouwsluis, Leiderdorp; in the river Gouwe: north of Gouda and near Gouwsluis, in the waterworks of Rotterdam, in the waterworks Lek en IJssel at Lekkerkerk, in the Kil waterworks at 's-Gravendeel, Snellerak on the Hollandsche IJssel, in the river Linge at Leerdam.

Part Z VI: Heusden on the river Maas, the Zuid-Willemsvaart, in the river Maas above Nierskanaal.

(Material in ML, MA, coll. A, Zoologisch Laboratorium Amsterdam.)

Often found together with Plumatellidae and *Christatella mucedo* Cuvier; not always growing on a special substratum as pilework, dead branches, Nuphar but often lying loose, growing among the zoaria of other fresh-water Bryozoa or in mud. The zoaria vary in length, the walls of the zooecia are as clear as glass as a rule but sometimes incrustated. Among the material at my disposal and gathered from 26th May until 27th September there is only one zoarium with hibernacles, elongated and spindle-shaped, from Sonsbeek, August 7th 1942.

Cl 30-1719 mg/l, the last named number in brackish water near Halfweg. pH 7.2-8.42. H_2CO_3 0.274-0.291 o/oo.

Among the material of *Alcyonidium* from various localities there were some zoaria with deviating characteristics, determined as *Alcyonidium gelatinosum* (L.), much resembling that species but not belonging to it. I propose a new name for them: *Alcyonidium proliferans* species nova. Pl. XIII, fig. 2; Pl. XV; Fig. 2; Fig. 3 C and D; Fig. 5 C.

Diagnosis:

zoarium: an erect stem, robust, with a thin proximal part, not or sparingly branched, rounded or flattened in section, set with knobs and lobes; colour greenish-brown;

zoecia: concentrically disposed in the flat parts of the zoarium and spread out, in the knobs crowded together; distally broadly rounded, proximally narrowed, with an orificium that is surrounded by an annulus; cuticula and septa very thick. Measurements on an average: length $481\ \mu$, breadth $298\ \mu$. The polypide is slender, the number of tentacles 14-16; intertentacular organ perceived.

Localities: I: de Haart on the island of Schouwen (ML.).

II: Terheide (ML), Scheveningen (MA), Katwijk (coll. A, ML), Noordwijk (ML), IJmuiden (MA). Horn Reef (MA), Leman Grounds (coll. A).

Distribution as far as known: Northsea.

Type-specimen: in coll. A, Katwijk, 27 XI 1938.

The zoaria are robust, the greatest height known to me is 27 cm, the surface is nodulated. The species is not rare but was probably overlooked.



Fig. 2. *Alcyonidium proliferans* sp. nov. outlines of zoarium;
a: from Scheveningen,
b: from Leman Grounds.

A comparison of this species which has 14-16 tentacles with *Alcyonidium gelatinosum* which has 15-17 tentacles and *Alcyonidium hirsutum* which has 17-18 tentacles shows distinct differences. In the new species the zoaria are pear-shaped, only autozooecea are present. In *A. gelatinosum* the autozooecea are irregularly hexagonal, separated by small furrows; small zooecea, which contain a rudiment of a polypide (kenozooecea) occur, lying scattered between the autozooecea. In *A. hirsutum* the autozooecea are surrounded by papillae, which also contain elements of a polypide, visible after staining by eosine and haematoxyline.

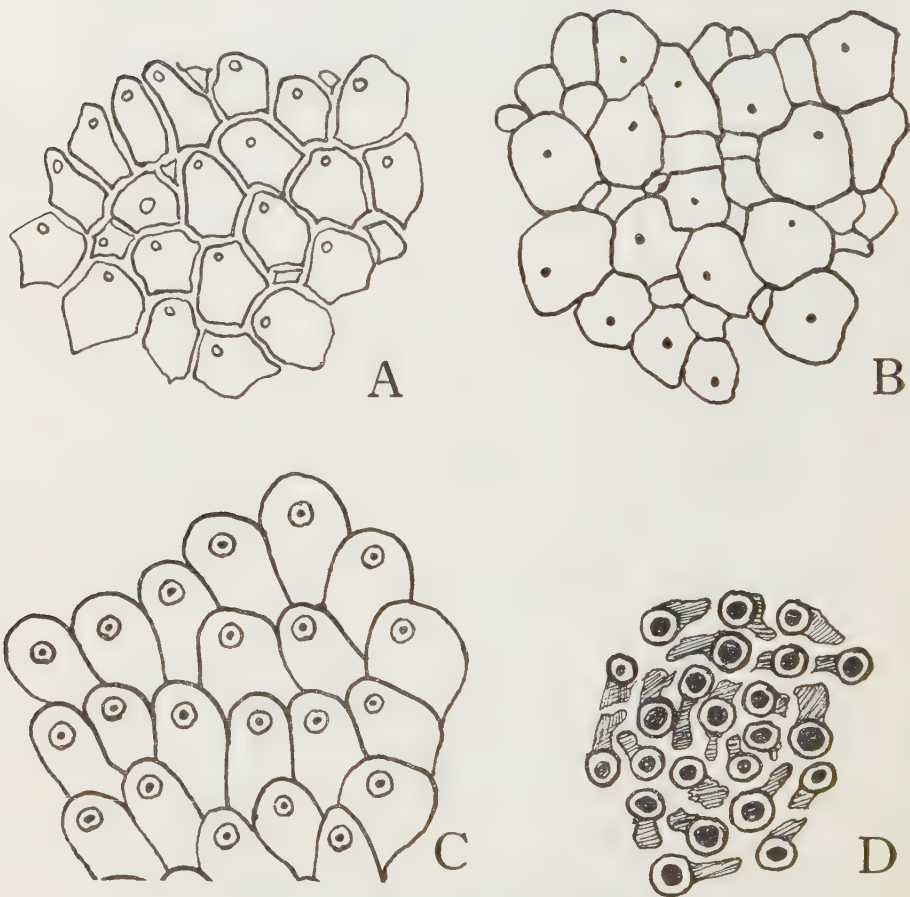


Fig. 3. Form of the zooecea:
 A *Alcyonidium gelatinosum* (L.)
 B „ *hirsutum* (Fleming),
 C, D „ *proliferans* sp. nov.

The microscopical section of the stem shows that the centre is composed of vacuoles, which are invested by epithelium. These

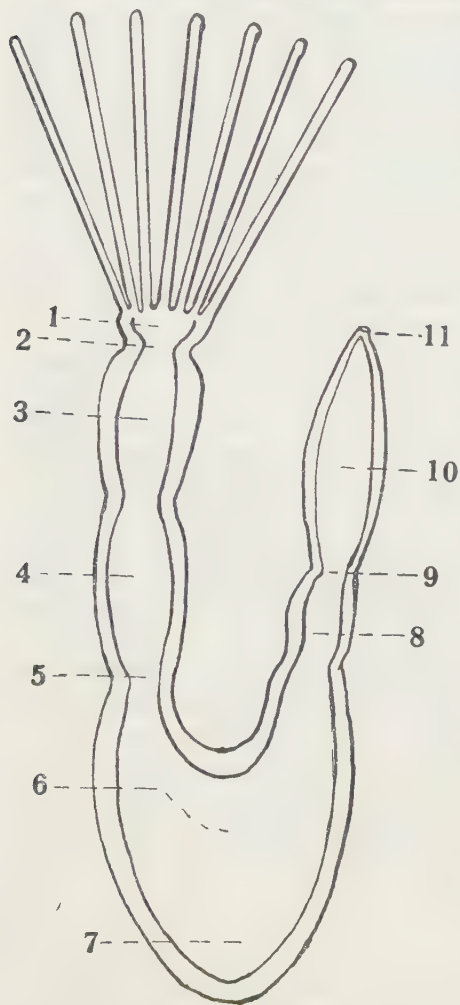


Fig. 4. Alimentary canal of the Bryozoa, schematically drawn.
(Explanation in the text)

vacuoles originate from the zooecia which come to lie centrally by budding on the periphery. In the outer layer the auto-zooecia are found. In the new species they are narrow, the vacuoles are small, the polypide is tall and long, the cuticula and the septa are very thick, the centre and the outer layer are not distinctly separated but merge into each other. It appears that the zooecia in *A. gelatinosum* are much more spacious, the vacuoles are large also; the polypide is big, the cuticula and the septa are thin; the centre and the outer layer are distinctly separated. In *A. hirsutum* the zooecia are about half as spacious as in *A. gelatinosum*, the vacuoles are rather broad but flat; the polypide is less big, the cuticula and the septa are thin; the centre and the outer layer are not distinctly separated.

Before I compare the alimentary canal of the three species, some re-

marks on this organ must be made.

Several authors have called the same parts by different names. So the under part of the stomach (7) is named "blind sack" or "colon". As the colon is properly speaking a part of the intestine

that leads from the stomach to the anus, it is not possible that a part of the stomach should be "colon". The last part of the stomach (8) is also often named "colon" but this is not correct either. The ventriculus of the Phylactolaemata shows lines of cells, running from the cardia (5) to the bottom and from the bottom to the pylorus (9); so the last part (8) which is slightly narrowed belongs to the ventriculus. ANNANDALE (1916) calls the upper half of the pharynx "pharynx" and the lower half "oesophagus"; how the oesophagus proper ought to be named he does not mention. The gizzard of the Ctenostomata is a modification of the oesophagus. CORI (1941) has copied several figures and erroneous denominations, given by other authors, also that by ANNANDALE, without criticism¹).

As it is necessary to use a uniform nomenclature, I propose the following: the lower part of the funnel, formed by the tentacles is the infundibulum tentaculorum (1), the opening in this infundibulum, the "mouth": ora (2), the food passes through this ora into the pharynx (3) and the oesophagus (4) and through the cardia (5) into the ventriculus (6). The cardia (5) is no more than a constriction, so it has but very small dimensions. One may speak of a cardiac part of the ventriculus, though it does not exist physiologically; one could mention it on account of an anatomical peculiarity, the musculature. I call the "blind sack" fundus ventriculi (7) and the last part of the ventriculus that is slightly narrowed, the antrum pylori (8); then the pylorus (9) which too is but a constriction and is also of very small dimensions like the cardia; finally the rectum (10), closed by the anus (11).

Now comparing the three species in question, it appears that distinct differences exist, clearly shown by the figures.

¹) The legends of some figures are also wrong: fig. 350 is not *Flustrella hispida* (Fabr.) but *Alcyonidium albidum* Alder; fig. 429, 432 and 433: *Plumatella princeps varietas fungosa* is now named: *Plumatella fungosa* (Pallas).

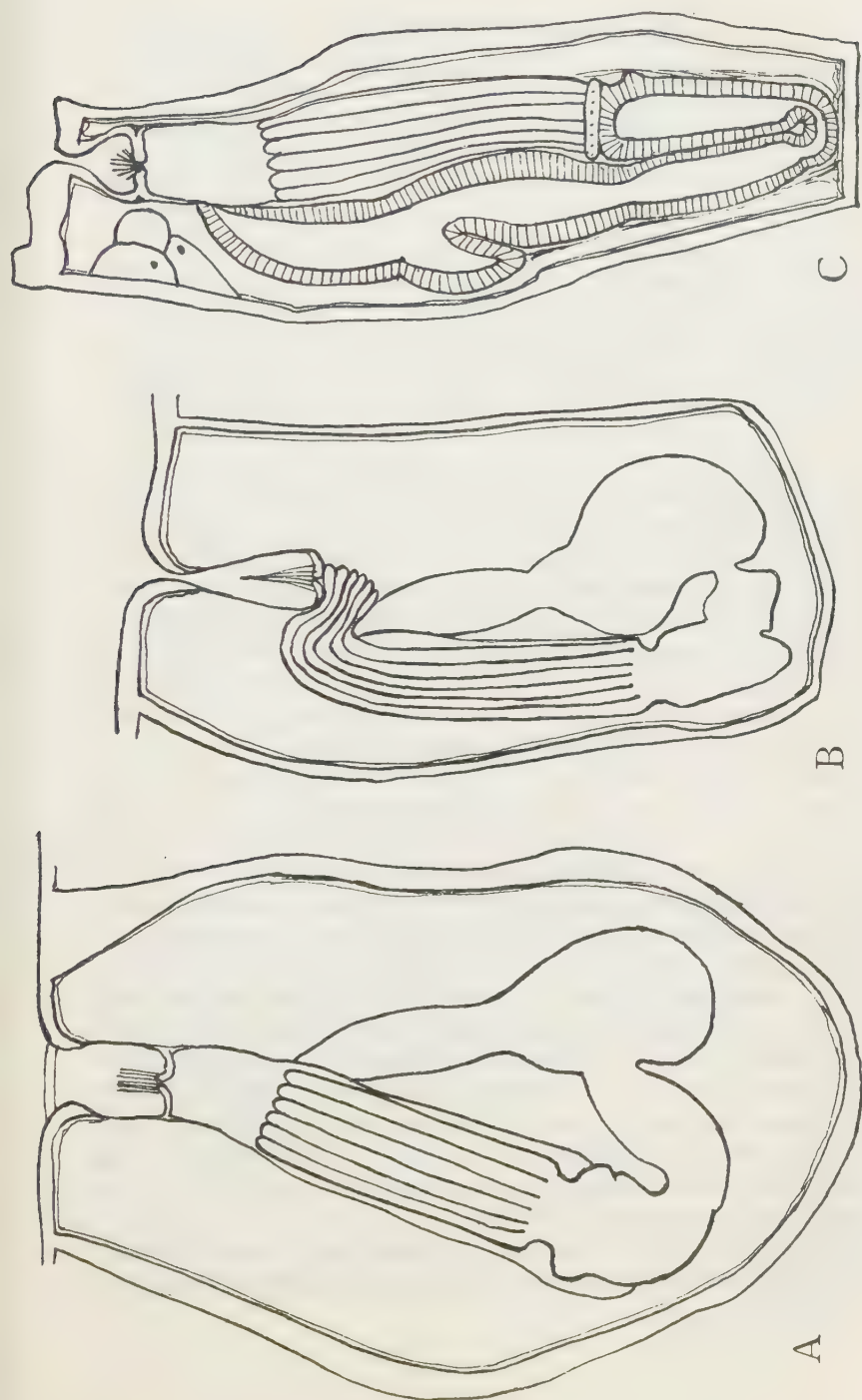


Fig. 5. Alimentary canal:
 A *Alcyonidium gelatinosum* (L.);
 B " *hirsutum* (Fleming);
 C " *proliferans* sp. nov.

	<i>A. gelatinosum</i>	<i>A. hirsutum</i>	<i>A. proliferans</i>
vestibulum:	broad and rather long,	pear-shaped, long,	broad and short,
diaphragma:	furnished with short setae, parallelly directed,	furnished with long setae, convergently directed,	furnished with very short setae, divergently directed,
tentacles:	short,	very long,	long,
pharynx:	broad and short,	short and of small proportion	narrow and long
oesophagus:	wide and long,	narrow and short,	narrow and long,
vertriculus:	large and sack-shaped,	very large, bulky,	not so large, with pointed fundus,

On account of the small number of tentacles I place *A. proliferans* before *A. gelatinosum*.

I have compiled a list of 71 marine species; the localities which have been mentioned, are taken from the material and the literature; that is to say that the species have been found there, that it is not certain that they are still living there or that they have lived there and that it is possible that some statements are wrong. 44 species are indigenous. I have noticed in what parts they have been found. The other species have reached the Dutch shore on all kinds of substratum, they belong to the "naturally adventitious element" in the fauna (VAN REGTEREN ALTENA Proefschrift 1937). This number is much liable to extension by means of passive displacement, specially from the south. These transported species need not always be regarded as having been removed from their region of distribution, for the Northsea belongs to that region, but they are not indigenous to the Netherlands. Most species are known from the adjacent countries. The localities on the Dutch shore of some species lie more or less isolated; this is the case with *Crisia aculeata*, *Tubulipora phalangea*, *Hornera lichenoides*, *H. violacea*, *Lichenopora hispida*, *L. verrucaria*, *Aetea anguina*, *A. sica*, *Electra hastingiae*, *Acanthodesia tenuis*, *Tegella unicornis*, *Callopora lineata*, *Rhamphonotus minax*, *Schizoporella unicornis*, *Escharina spinifera*, *Hippodiplosia pertusa*, *Cellepora armata*, *Alcyonidium mamillatum*, *Bowerbankia caudata*, *Anguinella palmata*.

The small number of indigenous species is due to the unfavourable condition of the shore; but little steady substratum is present.

Moreover the knowledge of the Dutch Bryozoan-fauna is insufficient. Nearly all the marine material has been washed ashore; only little has been obtained by dredging. The small number of Cyclostomata 4 is striking; Cheilostomata 26, Ctenostomata 14. The last ordo is relatively most widely distributed, they are more able to adaptation in brackish water than the other species. In region I 34 species (resp. 3, 19, 12) were found; this is the region of the estuaries of Zeeland and Zuid Holland with favourable substratum in some places; from the waters round the islands of Zuid Holland nothing is known. In region II 37 species (resp. 2, 23, 12) were found. It contains the continental shore with den Helder-Nieuwediep as locality of some species; here *Flustra foliacea* (L.) is substratum for other Bryozoa as well as in the other regions; from the piers and breakwaters, "artificial rocks", in this part still nothing is known, except for den Helder: *Cryptosula pallasiana* (Moll) was found here on stones of the sea-dike. In region III only 13 species (resp. 1, 6, 6) were found; this region, including the Waddenzee, the Islands, the coast of Friesland and Groningen has been but little investigated; but there is few favourable substratum here. *Hypophorella expansa* Ehlers, living in worm-tubes, was found on the German shallows and may undoubtedly live here too.

B. FRESH-WATER BRYOZOA.

Ordo Phylactolaemata.

While the marine Bryozoa are represented only in small numbers, the fresh-water species do comparatively better; 7 out of 9 European species were found in the Netherlands. Since I was able to compare my material with foreign material I have to mention that my two new species *Plumatella wysteri* and *Plumatella serrulata* Lacourt (1944) must be dropped.

In order to have an impression of their distribution, I have divided the country into parts which are based upon the regions of FABER (Nederlandsche Landschappen, 1942), enumerated I-VI, with the indication "Z" to distinguish them from the marine regions. Part Z I, the northern part, covers Friesland en Groningen; part Z II Drente; part Z III, the eastern part, covers Overijssel and Gelderland east of the river IJssel; part Z IV, the central part, covers Gelderland west of the river IJssel, Utrecht

and Gooiland; part Z V, the western part, covers Zeeland, Zuid Holland, Noord Holland minus Gooiland but including the Wadden Islands; part Z VI, the southern part, covers Noord Brabant and Limburg.

Fredericella sultana (Blumenbach).

Pl. XVI, fig. 1; BORG 1930, p. 111, fig. 151-153.

Z II: Drentsche A near De Punt: on the stakes of the boat-house and on the inlet of the waterworks, Leisloot between Eemster and Dwingelo'sche vaart near the weir on bricks under a bridge (both in coll. A. ML).

Z IV: Arnhem-Sonsbeek, (HOFKER 1928), (coll. A).

Z V: Roelofarendsveen, Rotterdam waterworks (statoblasts), waterworks Lek en IJssel at Lekkerkerk, Kil-waterworks at 's-Gravendeel (all in coll. A), (ML).

This species possesses only fixed statoblasts; measurements: length 398-498 μ , breadth 249-298 μ .

Plumatella fruticosa Allman.

Pl. XVII, fig. 2-3. BORG 1941, p. 485, Taf. I, fig. 1-2.

Z II: an old branch of the Beiler stroom near Lheebroek in the province of Drente on Nuphar and near the small weir in the Dwingelo'sche stroom (the same river but downstream) on Nuphar, (coll. A).

Measurements of the fixed statoblasts: length 697 μ , breadth 265 μ ; of the free statoblasts: length 520-600 μ , breadth 200-249 μ .

Plumatella emarginata Allman.

Pl. XVI, fig. 2: BORG 1941, p. 488, Taf. I, fig. 3-4.

Plumatella lucifuga (Vaucher), DE VRIES 1887.

Z II: Drentsche A near de Punt: on the stakes of the boat-house of the waterworks, (coll. A), (ML).

Z V: Rotterdam waterworks, Kil-waterworks at 's-Gravendeel, waterworks Lek en IJssel at Lekkerkerk (all in coll. A), (ML).

The fixed statoblasts are rare; they are found in the basal parts of the tendrils only and will rest on the substratum when the zoarium is torn off. Measurements: length 500 μ , breadth 380 μ . The free statoblasts are abundant; usually they are uniformly rounded oval in outline, more oval than in the figure by BORG (1941). Measurements: length 415-500 μ , breadth 230-265 μ .

Plumatella repens (L.).

Pl. XVI, fig. 3-4; BORG 1930, p. 116, fig. 149, 159-161.

DE VRIES 1887, MAITLAND 1897, POSTHUMA 1916, PEETERS 1919, DORSMAN 1919, HOFKER 1928.

Z II: waterpassage along Eelderwolderhooiweg, Drentsche A near De Punt, an old branch of the Beiler stroom near Lheebroek, old branches of the Dwingelo'sche stroom near the old lock.

Z III: a pond of the country-seat Stokhorst at Enschede, Buurser Beek, canal of the pumping-station Afsched.

Z IV: the river Waal at Nijmegen, Sonsbeek, Hurwenensche Kil near Zaltbommel, Lake of Abcoude, Gein and Holendrecht at Abcoude, in the river Vecht, Nieuwersluis, Spakenburg.

Z V: Geldersche Hoek in the province of Noord Holland, in the environs of Amsterdam, Ringvaart of the Haarlemmermeerpolder, a number of localities in the northern part of the province of Zuid Holland, Rotterdam waterworks.

Z VI: Nieuw Wiel overflow at Drunen. (ML, MA, Zoologisch Laboratorium Amsterdam, ROMIJN in archives, DE Vos in litt.). Specially on *Nymphaea* leaves, also on floating beams, treebark, bricks. Occasionally this species occurs in brackish water also, but far less than the following. It lives under different conditions: Cl 1.5-261 mg/l. pH 7.3-7.8, H_2CO_3 0.034-0.183 o/oo.

Measurements of the fixed statoblasts: length 560-600 μ , breadth 430-515 μ ; of the free statoblasts: length 350-380 μ , breadth 265-280 μ .

Plumatella fungosa (Pallas).

P. XVII, fig. 1; BORG 1930, p. 117, fig. 162-163.

BENNET EN VAN OLIVIER 1826, MAITLAND 1851 and 1897, DOCTERS VAN LEEUWEN 1912, POSTHUMA 1916, BLAAUW 1917, ROMIJN 1918, DORSMAN 1919, OTTO 1927, HOFKER 1928, OTTO EN WIELINGA 1933.

Z I: in brackish water in the environs of Harlingen, Harlingen-harbour (statoblasts), Langweerderwielen (statoblasts of this species?), pond of the Hortus Botanicus at Groningen, van Starckenborchkanaal near railway-bridge near Dorkwerd.

Z II: waterpassage along Eelderwolderhooiweg, Hijkermeer, Friesche Veen.

Z IV: fish-ponds at Emst, Sonsbeek, Beek near Nijmegen, Oosterbeek-oorsprong, Roodvoet near Rijswijk in the Betuwe, Culemborg, in the river Vecht (statoblasts).

Z V: canals and environs of Amsterdam, harbour Oude Zeug in the Wieringermeerpolder, a number of localities throughout the provinces of Zuid Holland and Noord Holland, Rotterdam-

waterworks, Lake of Rockanje, some localities on the island of Beierland, waterworks Lek en IJssel at Lekkerkerk,

Z VI: in the river Maas on breakwater opposite to Heyen, Bergen op Zoom, Melanen at Bergen, Stein in the province of Limburg.

(ML, MA, RVO, Zoologisch Laboratorium Amsterdam, ROMIJN in archives, DE VOS in litt.).

On waterplants, roots, immersed branches, stakes, tree-bark, on a ship's hull, on living *Unio pictorum* (L.), *Anodonta piscinalis* (Nilsson) and *Dreissena polymorpha* (Pallas) round their siphon where the molluscs cause a current of water and food is carried on, on *Balanus*.

This species can live very well in slightly brackish water. Some chemical conditions are known for this species: Cl 1.5-261 mg/l, pH 7-8, H_2CO_3 0.183-0.264 o/oo.

I have found the fixed statoblasts in the basal parts of the tendrils only; measurements: length 480-610 μ , breadth 365-500 μ . The free statoblasts of the material at my disposal show a peculiarity. In one case they are as small as those of *P. repens*. The measurements of these different statoblasts are given in a table.

statoblasts	small form	normal form
length:	320 μ	380-465 μ
breadth:	249 μ	233-330 μ

Plumatella fungosa is the most common fresh-water species in the Netherlands, contrary to the other countries of Europe where *Plumatella repens* is regarded as the most common.

Plumatella (Hyalinella) punctata Hancock.

BORG 1930, p. 119, fig. 164-166; MARCUS 1940, p. 374, fig. 216.

Z V: Kilwaterworks at 's-Gravendeel, waterworks Dubbeldam. (Always at the inlets of all waterworks here mentioned.) Oostkapelle (ditch along Kalfhoeksche weg, brackish water).

Free statoblasts were present in two dimensions in the same zoarium. The measurements of these different statoblasts are given:

	large form	small form
length:	431-464 μ	298 μ
breadth:	282-298 μ	232-249 μ

Lophopus cristallinus (Pallas).

BORG 1930, p. 120, fig. 167-169.

Z IV: Sonsbeek (HOFKER 1928).

Z V: Sorgvliet (TREMBLEY 1744).

Z VI: Haelensche beek (REDEKE 1932).

Cristatella mucedo Cuvier.

BORG 1930, p. 125, fig. 172-175.

MAITLAND 1897, BLAAUW 1912 and 1916, OTTO 1927 and 1930, HOFKER 1928.

Z I: Paterswoldsche meer.

Z II: waterpassage along Elderwolderhooiweg, an old branch of the Beilerstroom at Lheebroek (statoblast).

Z III: Zwarte water at Hasselt, Enschede.

Z IV: Apeldoornsch kanaal (statoblast), Sonsbeek, Oosterbeek: brook before the waterfall, Betuwe, the river Rhine at Wageningen: many statoblasts washed on the bank underneath the Wageningsche berg (BEIJERINCK in litt.), Holendrecht at Abcoude, in the river Vecht (statoblasts), Loenerveensche plas, Loosdrechtsche plassen.

Z V: Diepsmeer in the province of Noord Holland, Vrijenhoef and Nieuwenbroek near Gouda.

Z VI: Doode Maas at Giesen, Wiel on the road west of Terheyden, Lunet before Kraayenstein at Vught, Zuid Willemsvaart, Maas above Nierskanaal. In all the localities in Z VI only statoblasts.

(ML, Zoologisch Laboratorium Leiden and Amsterdam, ROMIJN in archives, DE Vos in litt.).

On water-plants, bricks, immersed branches, stakes. Cl 180 mg/l.

Measurements of statoblasts in diameter: 710-780 μ , the marginal spines on the dorsal side are long 150 μ ; they are on the ventral side mostly long 130 μ , they may reach 180 μ .

The fresh-water Bryozoa occur almost throughout the whole country; if their occurrence in some parts has not yet been stated, it is due to our insufficient knowledge in the first place. They live in clear, deep water, only by way of exception in small ponds or pools. In regions where brackish water is found Phylactolaemata occur far less or not at all; here *Electra crustulenta* predominates. I figure the statoblasts of 5 species by means of photomicrographs of my own, enlarged 120 $\times$. BORG (1941) represents photomicrographs of the statoblasts of *Plumatella fruticosa* and *Plumatella*

emarginata. I do not know others from literature. I figure the free statoblast of *Plumatella emarginata* with the oval form which is the most common in this country as far as known.

APPENDIX

After this article has been written a paper by BLOKLANDER EN LEENHOUTS (1948) has appeared. They record a number of species of which 11 are new for the Dutch fauna, the total number of species now known from the Netherlands bringing up to 90. The new species are: *Filicrisia geniculata* (M. Edwards), *Beania mirabilis* Johnston, *Caberea boryi* Audouin, *Membranipora* (*Callopora*) *dumerilii* (Audouin), *Scrupocellaria scrupea* Busk, *Haplopoma impressum* (Audouin), *Escharella variolosa* Johnston, *Rhynchozoon* (*Rhynchopora*) *bispinosum* Johnston, *Stephanosella biaperta* (Michelin), *Discopora* (*Umbonula*) *littoralis* Hastings, *Porella concinna* (Busk).

All species have been found on floating substratum.

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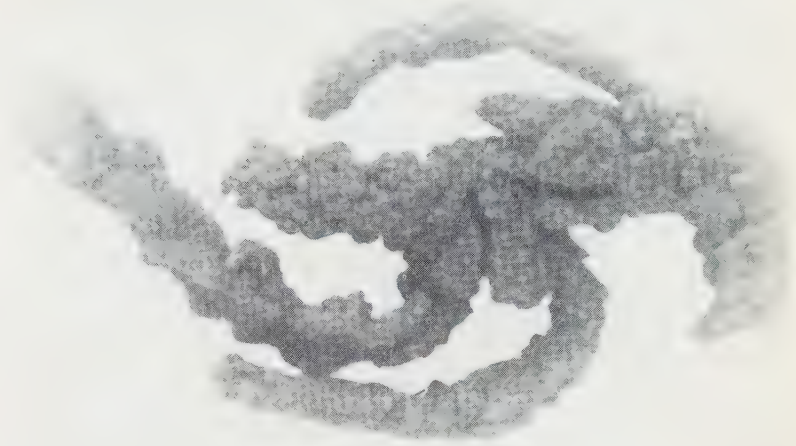
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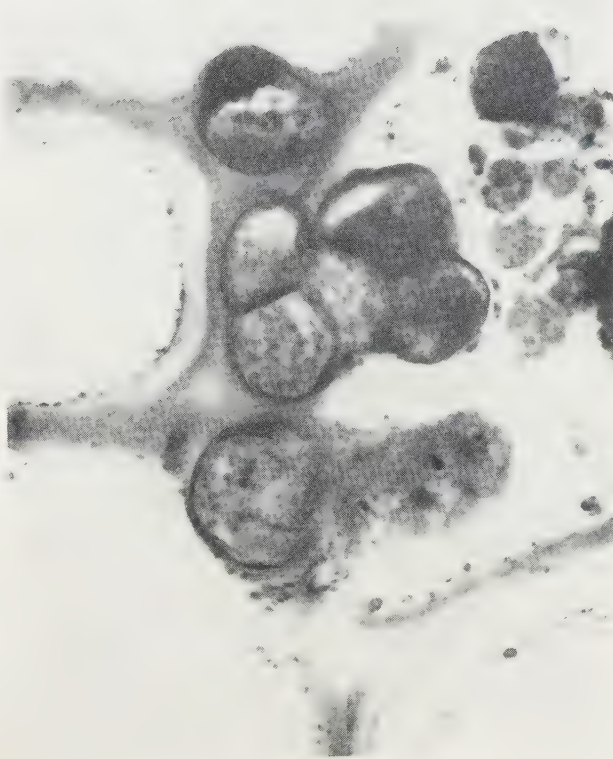
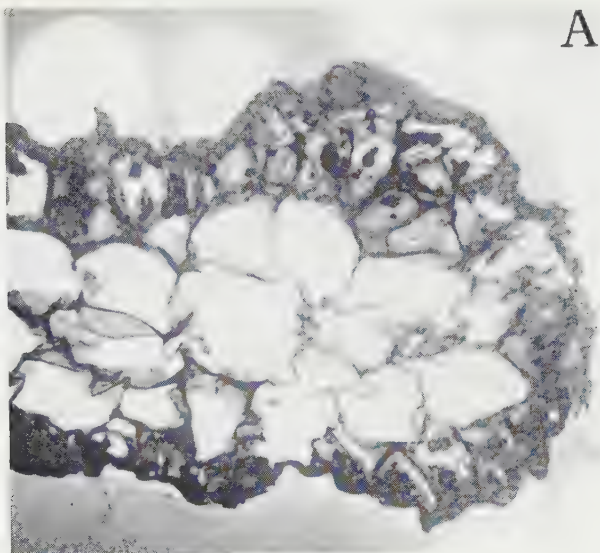
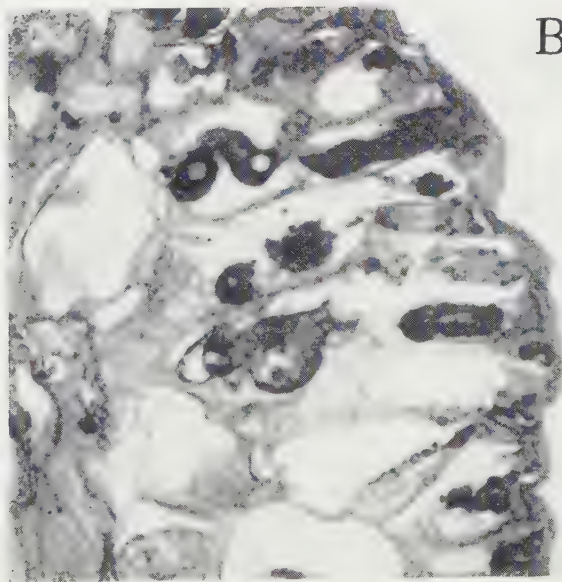


Fig. 1. Foreign bodies in the septa of *Alcyonidium gelatinosum* (L.), which cause a brownish-violet colour of the zoarium.
(Parasitic Protozoa or Algae?).

Fig. 2. *Alcyonidium proliferans* sp. nov., the type-zoarium.

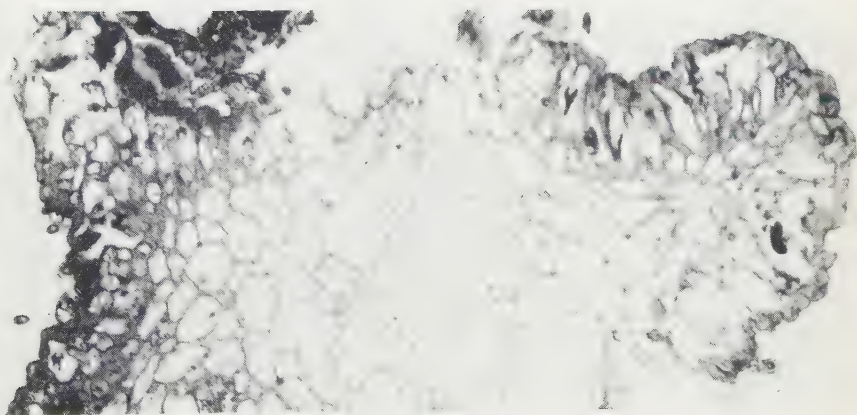
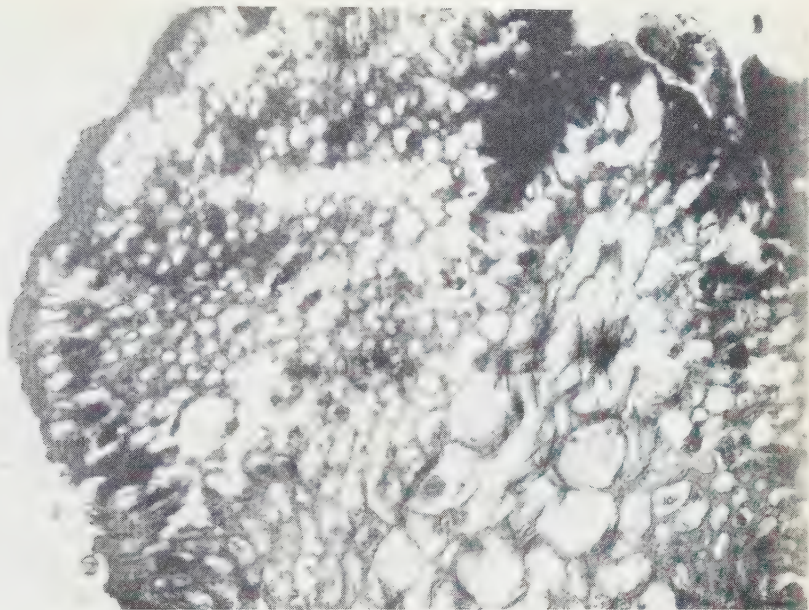


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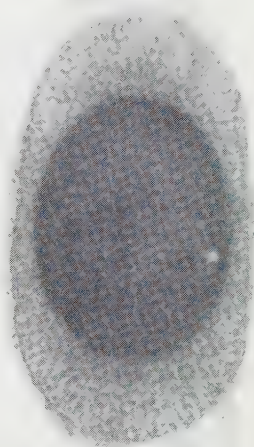
Microscopical sections of the stem of:
A *Alcyonidium gelatinosum* (L.);
B *Alcyonidium hirsutum* (Fleming).



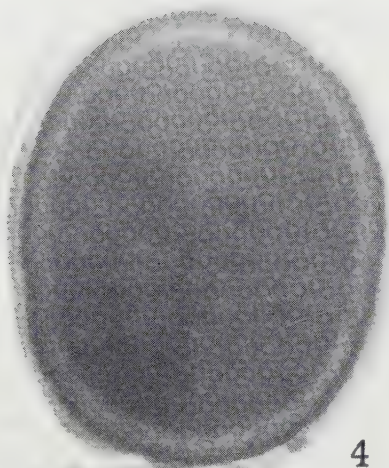
Microscopical sections of the stem of *Alcyonidium proliferans* sp. nov.



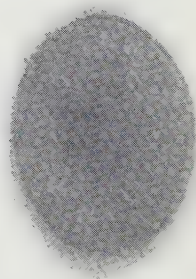
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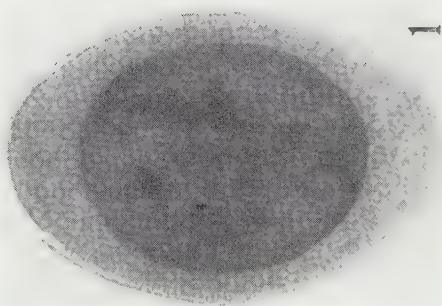


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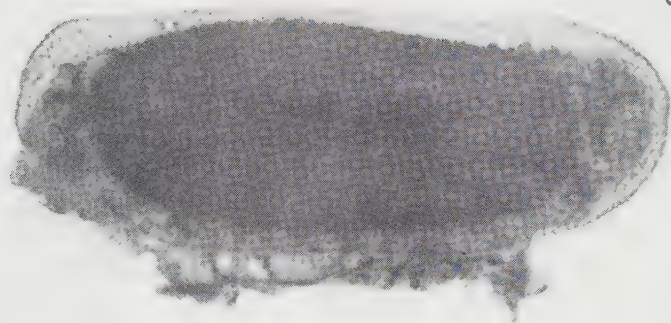


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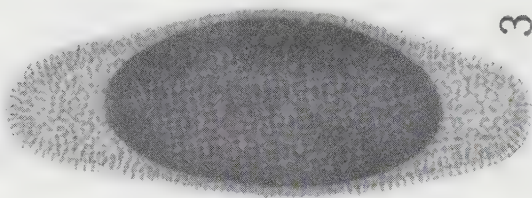
- Fig. 1. Statoblast of *Fredericella sultana* (Blumenbach);
 Fig. 2. Free statoblast of *Plumatella emarginata* Allman;
 Fig. 3. Free statoblast of *Plumatella repens* (L.);
 Fig. 4. Fixed statoblast of *Plumatella repens* (L.).



1



2



3

Fig. 1. Free statoblast of *Plumatella fungosa* (Pallas).

Fig. 2. Fixed statoblast of *Plumatella fruticosa* (Allman).

Fig. 3. Free statoblast of *Plumatella fruticosa* (Allman).

ON THE STRUCTURE OF CYCLOPIC,
SYNOPHTHALMIC AND ANOPHTHALMIC EMBRYOS,
OBTAINED BY THE ACTION OF LITHIUM
IN LIMNAEA STAGNALIS

BY

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When eggs of *Limnaea stagnalis* are exposed, during the period of development between the oviposition and the 24-cell stage, to solutions of lithium chloride of 0.01% to 0.001%, various head malformations are produced in a certain percentage of cases (RAVEN 1942). As a rule, a graded series of malformations is obtained, ranging from nearly normal young snails to highly teratological forms. In the lightest cases, the dorsal part of the head is somewhat narrowed, and the eyes, which are placed wide apart in normal embryos, have approached each other dorsally. This may be accompanied by a splitting of one of the eyes, so that triophthalmic forms arise. In other cases, one of the eyes and tentacles is reduced or absent: monophthalmia asymmetrica. With increase of the effects of the Li-treatment, the eyes meet each other in the median plane of the head: synophthalmia dorsalis. These cases lead over to the embryos with true cyclopia, where a single eye is situated dorsally in the median plane of the head. Still further reduction of the head region brings about anophthalmic embryos, where the head consists of a small knob projecting above the foot. Even this may be absent or nearly so: acephalic embryos. In all these cases, the reduction process affects the head region only: the foot and the trunk part of the body are normal or only slightly affected. Only in a small percentage of cases highly abnormal forms arise, in which the relation between the different parts of the body is seriously disturbed.

The observation that one can produce cyclopia by lithium in *Limnaea*, as in the Amphibia according to LEPLAT (1919), ADELMANN (1934) and LEHMANN (1936), is of great interest. It is well known that in Amphibia cyclopia is not produced by a direct influence of lithium on the eye rudiments, but rather in-

directly by its action on the archenteron roof. In Mollusks, nothing is known concerning an interaction between different organs of the embryo, comparable to the embryonic induction known in Amphibia. It seemed probable, therefore, that only a superficial resemblance existed between the malformations obtained in both groups, and that their mode of origin was in reality quite different. A detailed study of the embryos with head malformations was needed to answer this question.

RANZI (1929) obtained malformations of the cyclopic series by the action of lithium on the eggs of *Loligo vulgaris*. They resemble in many respects the malformations found in *Limnaea*; in other points, however, the results obtained in both groups cannot be compared. In *Loligo* the cyclopic malformations are often accompanied with a defective development of the stomodaeum, the statocysts and various other organs: in *Limnaea*, they present a much more uniform picture.

MATERIAL AND METHODS

42 cyclopic, synophthalmic, triophthalmic, anophthalmic and acephalic embryos have been studied. They were obtained in my lithium cultures in 1942 and 1944. Details on the method of treatment have been given in former papers (RAVEN 1942; RAVEN, KLOEK, KUIPER and DE JONG 1947).

The embryos were fixed partly in formol, partly in BOUIN's fluid; the latter fixation gave better results. They were sectioned at $7\frac{1}{2}\mu$, and the sections stained with iron haematoxylin and eosin or with azan.

THE NORMAL EMBRYO

1. General survey of the development (fig. 1)

Till the gastrula stage, the embryo of *Limnaea* preserves a nearly radial structure. The archenteron is invaginated at the vegetative pole (fig. 1¹). From this moment on, however, the posterior half of the embryo shows a more rapid growth, in consequence of the swelling of the head vesicle (h.v.), the growth of the visceral mass and the formation of the foot (f). In consequence of this, the original animal and vegetative pole approach one another on the anterior side, the egg axis is bent.

At the trochophore stage (fig. 1²⁻³), the mouth, which derives

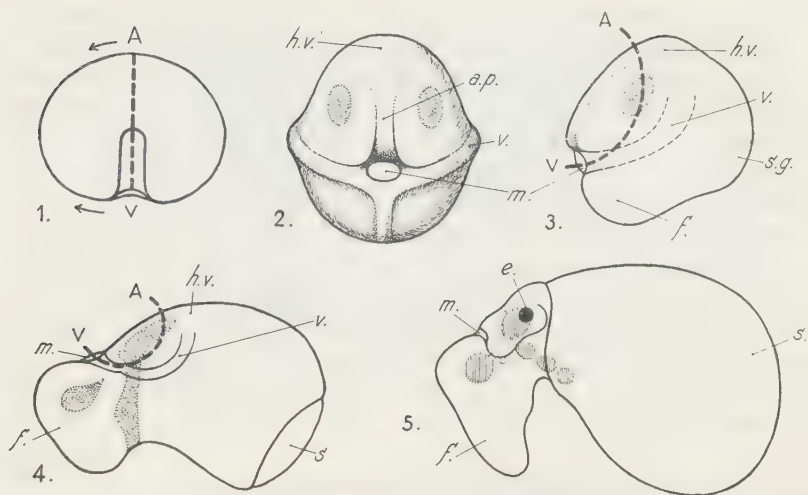


Fig. 1. *Limnaea stagnalis*. Normal development. 1. Gastrula. 2-3. Trochophore. 4. "Veliger". 5. Hippo-stage. Nr. 2 from the anterior side, the other figures from the left side. A = animal, V = vegetative pole. a.p. apical plate. e. eye. f. foot. h.v. head vesicle. m. mouth. s. shell. s.g. shell gland. v. velum.

from the blastopore, is situated on the anterior side of the embryo. Four bands of large ciliary cells meet here: a median band of the foot, the 2 halves of the prototroch or velum (v), and the apical plate (a.p.). The latter runs in the direction of the animal pole, where it meets the head vesicle (h.v.), which forms a transverse girdle of big swollen cells; this is continuous on either side with the posterior ends of the velum. The apical plate, head vesicle and velum surround two fields consisting of small ectoderm cells, representing the common rudiments of the cerebral ganglia, the eyes and tentacles.

At a later stage (fig. 1⁴), the head region has become relatively smaller. The foot (f) has now been delimited by a distinct groove. In the stippled regions, the ectoderm shows a proliferation which gives rise to the ganglia.

At about 10 days, a stage is reached in which all parts of the adult are recognizable (fig. 1⁵). It is this stage which will be described in greater detail below.

2. External appearance

Plate XVIII fig. 1 shows the external appearance of this stage.

The head, foot and visceral mass can easily be distinguished; the latter is entirely enclosed within the shell, which is beginning to coil. In the head region, the lateral tentacles are prominent hemispherical outgrowths. At their base, the eyes are showing as dark spots through the ectoderm; they are wide apart at the sides of the head. The mouth is situated in a groove at the boundary between head and foot; it is bounded on both sides by somewhat prominent lip-tentacles.

The shape of the foot is very variable. In the living embryo, however, it is worn, as a rule, in such a way that the head, with its "bulging" eyes, and the foot of the embryo together bear an unmistakable resemblance to the head of a hippopotamus (cf. Plate XVIII fig. 1b). It is called, therefore, the "hippo-stage" in the jargon of our laboratory.

3. The structure of the head.

a. Nervous system Fig. 2 shows a diagrammatic representation of the nervous system of an advanced "hippo-stage" embryo, seen from the dorsal and the right side. It is composed of 11 ganglia. The cerebral ganglia, situated on either side of the buccopharyngeal mass, are connected on the dorsal side of this mass by the cerebral commissure. The cerebropedal connectives join them to the pedal ganglia, which are situated in the foot ventrolaterally to the radular sac and are connected with each other by the pedal commissure. The eyes are found against the dorsolateral sides of the cerebral ganglia, the statocysts behind the cerebropedal connectives. The buccal ganglia lie against the posterior side of the buccopharyngeal mass, in the angle beneath the origin of the oesophagus; they are connected to the cerebral ganglia by the buccal connectives. Finally, there is a chain of 5 ganglia, united by a horse-shoe-shaped commissure. From left to right, it contains successively: the left pleural ganglion, the parietal ganglion, the abdominal ganglion, the suprainestinal ganglion and the right pleural ganglion. Several nerves take their origin from these ganglia, with the exception of the pleural ganglia from which no nerves arise.

The ganglia consist of ganglion cells with big clear vesicular nuclei, surrounding the fiber tracts which form the central part of the ganglia. On the whole, the ganglia of one side are near together; especially in the early "hippo-stage", the cell masses of two adjacent ganglia are often continuous; at more advanced

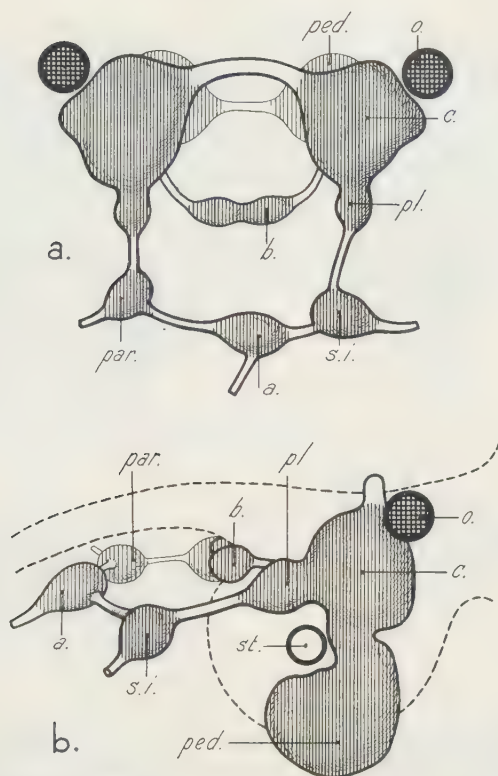


Fig. 2. Nervous system of advanced "hippo-stage" embryo, semi-diagrammatic. a. dorsal, b. right view. a. abdominal ganglion, b. buccal g., c. cerebral g., o. eye, par. parietal g., ped. pedal g., pl. pleural g., s.i. supra-intestinal g., st. statocyst.

stages, they are, however, more clearly delimited. The cerebral and pedal commissures are, on the other hand, well-differentiated at an early stage. Especially the cerebral commissure is long and slender (fig. 3, 4); it connects the cerebral ganglia of both sides as a naked fiber tract about $75\ \mu$ in length. As a matter of fact, it is surrounded by a layer of flat mesenchyme cells, but no ganglion cells are accompanying the commissure. The pedal commissure is much shorter, having a length of $10\text{--}20\ \mu$ only.

b. Eyes. The eyes are situated on both sides within the slightly prominent tentacle rudiments (fig. 3). They are nearly spherical vesicles (fig. 4). Their wall consists of two parts. About

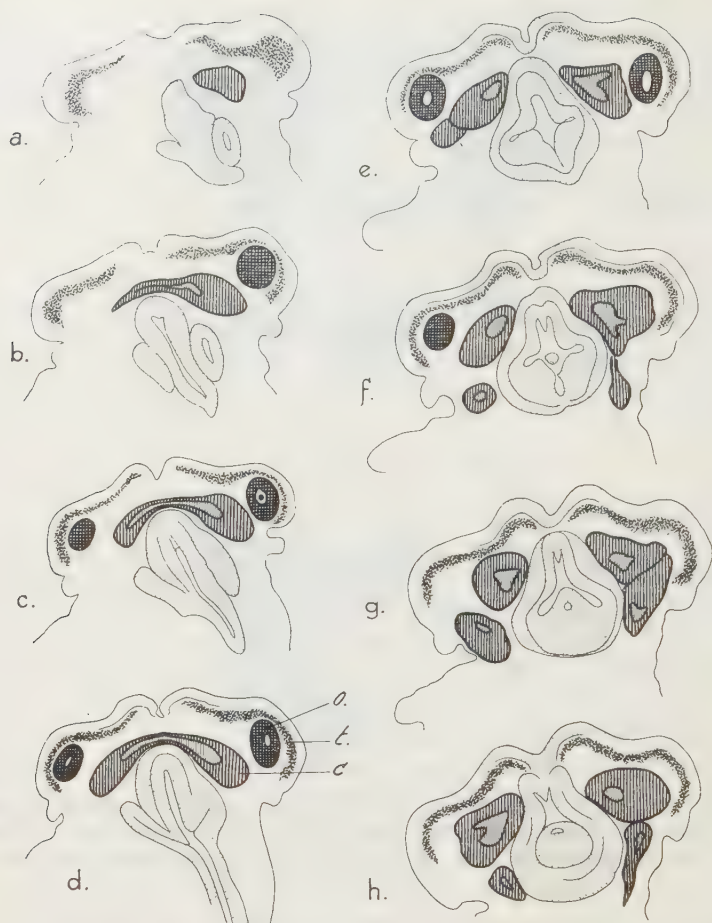


fig. 3. Series of transverse sections through head of normal "hippo-stage" embryo. *c.* cerebral ganglion. *o.* eye. *t.* tentacle field.

$\frac{3}{4}$ of the circumference is formed by the future retina, consisting of one layer of columnar to conical cells. The nuclei are situated in the outer part of these cells, whilst the inner part facing the lumen contains a great deal of brown-black pigment granules. The anterodorsal $\frac{1}{4}$ of the circumference of the eye consists of cuboidal cells with somewhat clearer nuclei and without pigment granules, forming the future inner corneal epithelium. The eyes contain a spherical lens, which stains deep-red with eosin.

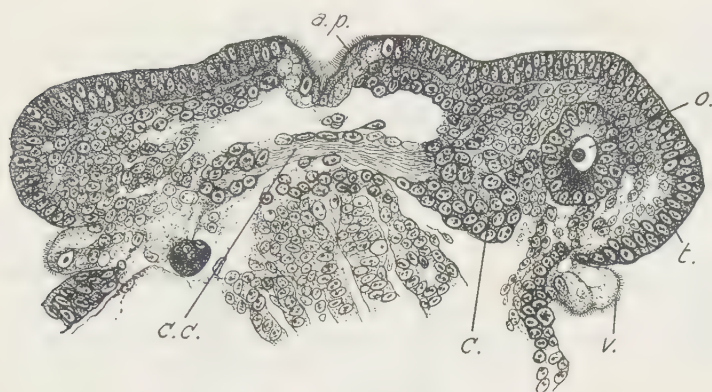


Fig. 4. Transverse section through the head, cf. fig. 4c. *a.p.* apical plate. *c.* cerebral ganglion. *c.c.* cerebral commissure. *o.* eye. *t.* tentacle field. *v.* velum.

c. Statocysts. The statocyst are nearly spherical vesicles, situated behind the cerebropedal connectives. Their wall consists of one layer of flattened cells with big clear vesicular nuclei and lightly-stained cytoplasm.

d. Nuchal cells. They form a group of giant cells, lying in the dorsal part of the head immediately behind the cerebral commissure. Dorsally this group is bordered by the posterior part of the apical plate and the head vesicle, ventrally by the pharynx and the first part of the oesophagus. The nuchal cells have large vesicular nuclei, with a peripheral chromatin network and a central or slightly eccentric nucleolus with clear internum. In their cytoplasm they possess a dense deeply-stained mass, which occupies the peripheral parts of the cell and exhibits a striped appearance. On the significance of these cells cf. BLOCH (1938).

e. Buccopharyngeal mass. The mouth leads into a bilaterally compressed mouth cavity, which is continued into the pharynx cavity. Along the dorsal wall of both cavities, a double row of ciliated cells with faintly stained protoplasm is found. From the dorso-lateral walls of the pharynx, the salivary glands arise; posteriorly, it is continued into the oesophagus. At the bottom of the pharynx, the radular sac opens with a narrow slit; towards its apex, this sac becomes wider and has a lumen which is crescent-shaped in transverse section. The radula is being formed from the anteroventral wall of the radular sac. Before this sac, a great fold is projecting into the pharynx lumen; it is the

rudiment of the tongue. This fold is filled with a dense mesenchyme, in which the formation of the tongue cartilage has begun. Anteriorly, the tongue is bounded by the paired sublingual cleft, whilst a deep suboesophageal cleft bounds the radular sac posteriorly. The whole buccopharyngeal mass is surrounded by a thick layer of mesenchyme.

f. *Tentacles*. On either side of the head, a thickened area of ectoderm is present (fig. 3, 4). The ectodermal cells in this field are elongated, columnar; since the nuclei are situated at different heights in the cells, one gets the impression of a stratified epithelium; in reality, however, the cells are arranged in one layer. Beneath the ectoderm, a dense mass of mesenchyme cells is present. The boundary between ectoderm and subepithelial mesenchyme is not quite sharp in all places. Perhaps, even at this stage a few cells may still be given off from the epithelium into deeper layers; no definite proof of this could be obtained, however. The lateral tentacles arise as hemispherical prominences from the dorsal part of this tentacle field. In the same way, the lip-tentacles are formed on either side of the mouth from the ventro-medial parts of this common field (fig. 3). Finally, near the posterior boundary of each tentacle field an invagination is present; they represent the so-called "cerebral tubes", which give rise to the accessory lobe of the cerebral ganglia.

g. *Velum*. The velum consists of a row of large cells, which are conspicuous by their size and their faintly stained cytoplasm. It begins on either side of the mouth, borders the tentacle field on the lateral and posterior side, and ends at the lateral angles of the head vesicle. Anteriorly, it consists of a single row of cells; posteriorly, on either side two giant cells, the anterior trochoblasts, are situated just above this row. These cells have a strongly vacuolated endoplasm and protrude considerably above the surface of the embryo. They bear but sparse cilia and the outer cell membrane is clearly marked; beneath this membrane, there is a narrow clear line, then follows a layer of strongly eosinophil ectoplasm. Between the vacuoles of the endoplasm granules can be observed. The nucleus is big, clear, vesicular and has a heavily stained nucleolus.

h. *Head vesicle*. The head vesicle forms a transverse girdle of 12 big swollen cells, lying in two rows one behind the other in the depth of the "neck fold", overhung by the anterior border of the mantle fold. Their endoplasm is strongly vacuolated and

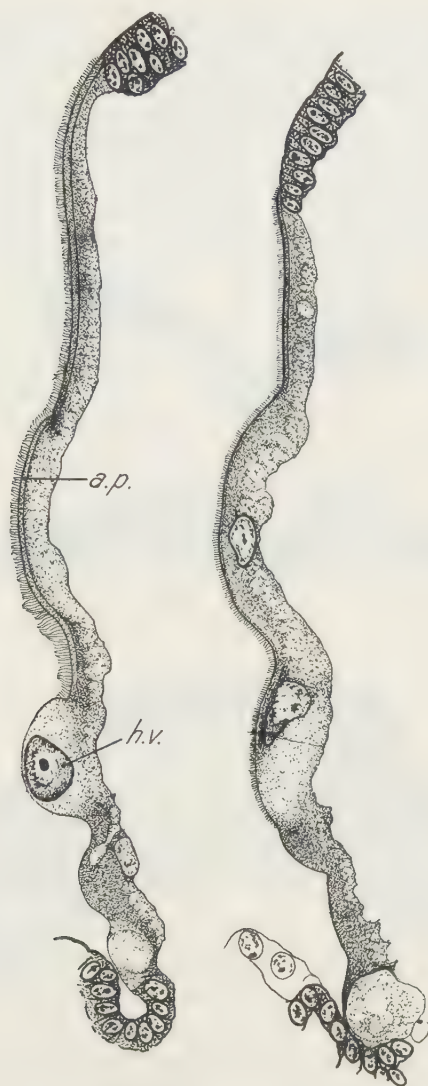


Fig. 5. Sagittal sections through the head of a normal embryo. Apical plate (*a.p.*) and head vesicle (*h.v.*)

the cells are nearly globular, protruding above the surface of the embryo (fig. 5). Fine granules are lying in the meshes between the vacuoles. The layer of ectoplasm is extremely thin, only in

the neighbourhood of the cell boundaries it is somewhat more conspicuous. The nuclei are large, clear, oval, with big heavily-stained nucleolus. The cells bear no cilia.

i. Apical plate. The apical plate extends in a sagittal direction from the mouth to the head vesicle, with which it is continuous behind; laterally, it is bounded by the tentacle fields of both sides. It consists of 7 flat ciliated cells (fig. 5, 6). The anterior cells are flattened very much. They possess a thin layer



Fig. 6. Transverse sections through the apical plate of a normal embryo.

of very dense eosinophil ectoplasm; the endoplasm is very clear and contains fine granules. The posterior cells are less thin; the layer of ectoplasm is less conspicuous than in the anterior cells; the granules in the endoplasm are somewhat coarser. The nuclei have the same character as those of velum and head vesicle, but, because of the flattening of the cells, they are somewhat more elongate.

CYCLOPIC EMBRYOS

Limnaea fQ 2: The embryo belongs to a batch with following history: Part of an egg-mass, containing 28 eggs, was put in a 0.001% LiCl solution, 7 hours after laying, when the eggs were

at the 8-cell stage. After 48 hours, at the gastrula stage, it was transferred to tap water and washed repeatedly. During the first 6 days, the embryos showed nothing abnormal, but on the 7th day, when the controls had developed to embryos with pulsating heart and twisted shell, the treated embryos proved to be all more or less abnormal, showing various degrees of head reduction. Altogether, the culture gave 6 cyclopic embryos (Plate XVIII fig. 2), 5 monophthalmic with failure of the left eye and tentacle, 3 anophthalmic, 5 acephalic and 4 teratomorphic embryos; 5 died before the type of malformation could be determined.

The embryo JQ 2 was fixed on the 9th day in BOUIN's fluid and sectioned transversely.

The first sections pass through the foot; the pedal ganglia are well-developed and symmetric; they are united by the pedal commissure, which has a normal appearance. The mouth is narrow; it is bordered by the oral tentacles. Immediately behind the mouth a median strip of flat ciliary cells is present, forming the anterior part of the apical plate. It has the normal width, and its cells have a normal appearance (fig. 7). However, only

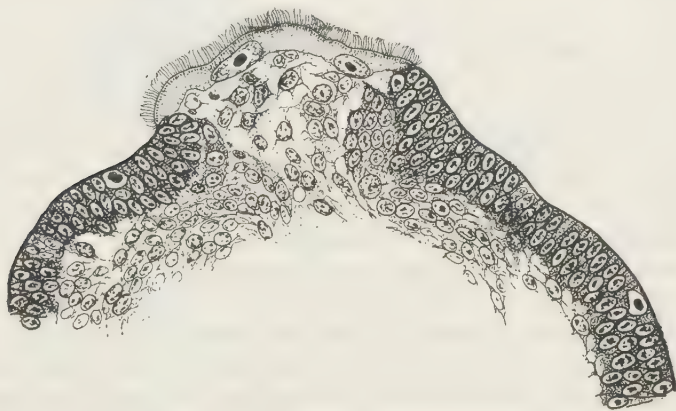


Fig. 7. *Limnaea* JQ 2. Transverse section through anterior part of apical plate and adjacent parts of tentacle field. Cf. fig. 8a.

2 nuclei can be seen in the apical plate (fig. 7) and it extends backwards over 7 sections of $7\frac{1}{2}\mu$ only, then it ends abruptly and the rudiments of the lateral tentacles unite to a single tentacle field (fig. 9); this extends backwards at the dorsal side of the head

for another 8 sections. Posteriorly, it is bordered by a transverse girdle of cells showing the typical appearance of head vesicle cells; lateroventrally, a single row of velum cells borders the tentacle field on both sides; they unite with the head vesicle posteriorly.

The buccal mass and radular sac are surrounded by a dense ring of ganglion cells; the individual ganglia are not yet clearly delimited. However, posteriorly two transverse connections can be distinguished, representing the visceral and buccal commissures, respectively. At both sides of the buccal mass the cerebral ganglia are situated. They are connected with the pedal ganglia by means of the cerebropedal connectives; the statocysts are situated medially to these connectives (fig. 8a). Anteriorly, the cerebral ganglia are fused into a single mass of ganglion cells; in its centre, it contains the cerebral commissure, consisting of a fiber tract which is surrounded by ganglion cells on all sides (fig. 8 d, e). Immediately behind this unpaired cerebral ganglion mass the cyclopic eye is situated; it forms a flattened vesicle with distinct pigment layer, containing a small lens (fig. 11), and lying exactly in the median plane of the head beneath the thickened ectoderm and mesenchyme layer of the unpaired tentacle field.

Summarizing, we can say that the nervous system is intact, but the cerebral ganglia have fused with one another to a single ganglionic mass surrounding the cerebral commissure. The velum, head vesicle and anterior 2 cells of the apical plate are present, but the posterior part of the apical plate is lacking; in this region, the rudiments of the lateral tentacles have fused into a single median tentacle field. Beneath this field a cyclopic eye is situated, which is well-differentiated.

Limnaea OW 3-II. An egg-mass, consisting of about 130 eggs, was put in a 0.001% LiCl solution, when the eggs were in a 12-16 cell stage. After 48 hours, it was transferred to tap water and washed thoroughly. 27 Embryos were fixed on the 3d and 4th days, as rather normal-looking but somewhat opaque larvae. On the 6th day, it became clear that several of the embryos showed various malformations. On the 7th day there were about 70 normal-looking embryos; 7 were cyclopic, 10 showed synophthalmic and triophthalmic malformations, 9 monophthalmia asymmetrica, 4 were anophthalmic and teratomorphic embryos. The cyclopic embryos were fixed on this day in Bouin.

OW 3-II was sectioned transversely. It shows a great resem-

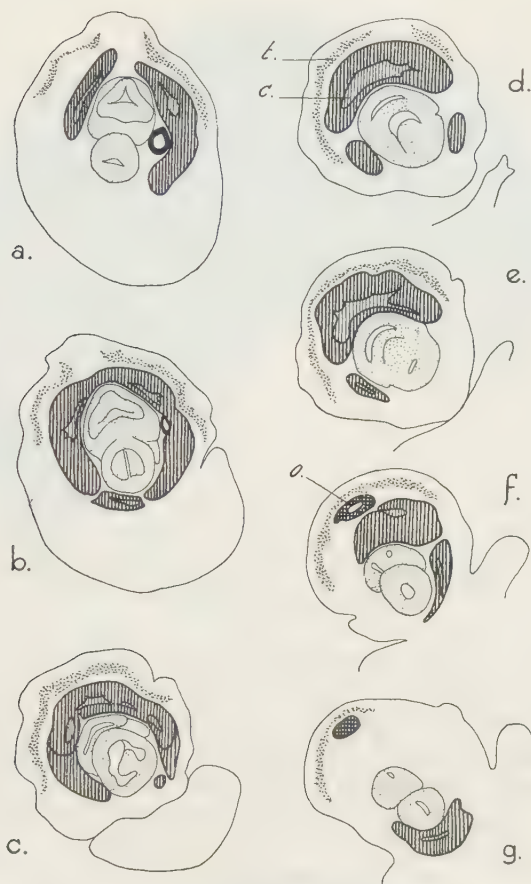


Fig. 8. *Limnaea* JQ 2. Series of transverse sections through the head. c. unpaired cerebral ganglion. o. cyclopic eye. t. unpaired tentacle field.

blance to the preceding case. As in the latter, the anterior part of the apical plate is present; it consists of 3 or 4 cells, which are ciliated but do not show the usual flattening (fig. 10); evidently, their differentiation is somewhat abnormal. Where the apical plate ends, the tentacle rudiments fuse into a single tentacle field (fig. 11). This field extends backwards over 17 sections of $7\frac{1}{2}\mu$, covering the dorsal side of the head; posteriorly, it is bordered by typical head vesicle cells; the velum is, however, not present.

The nervous system is intact; all the ganglia can be recognized. The cerebral ganglia have fused, however, in front of the buccal

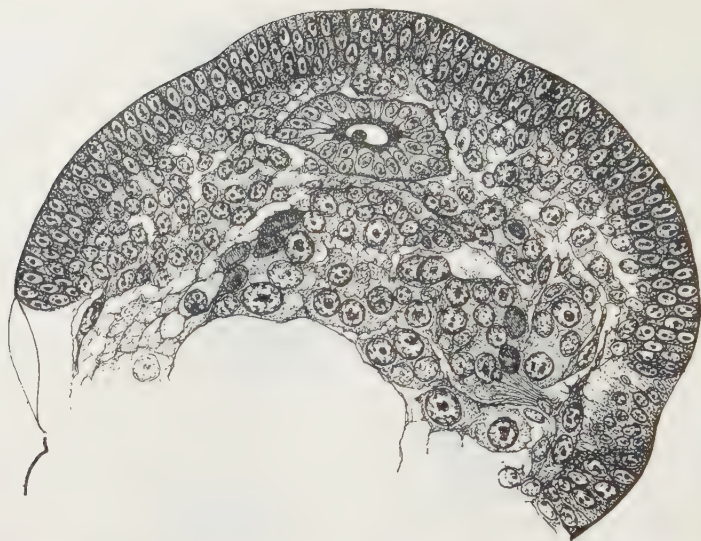


Fig. 9. *Limnaea* JQ 2. Transverse section through cyclopic eye and unpaired tentacle field. Cf. fig. 8f.

mass into a single unpaired ganglion which surrounds the cerebral commissure (fig. 12 c, d). The eye is situated before this unpaired cerebral ganglion mass, nearly exactly in the median plane of the head, beneath the unpaired tentacle field. It extends over 4 sections; in one of these, it shows an indication of reduplication, possessing 2 distinct lumina (fig. 12a). One of these con-



Fig. 10. *Limnaea* OW 3-II. Transverse section through anterior part of apical plate and adjacent parts of tentacle field.

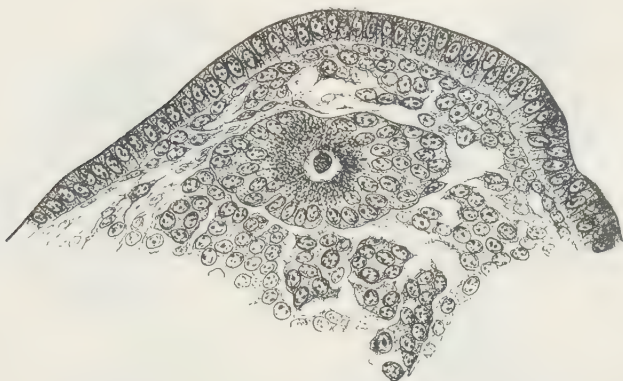


Fig. 11. *Limnaea* OW 3-II. Transverse section through cyclopic eye and unpaired tentacle field. Cf. fig. 12b.

tains a single well-developed lens (fig. 11); the other, which is smaller and lacks a pigment layer, contains 2 very small lentoids.

Summarizing, in this case the cerebral ganglia have fused to an unpaired ganglion mass surrounding the cerebral commissure; the head vesicle and anterior part of the apical plate are present, the velum and posterior part of the apical plate are lacking. The rudiments of the lateral tentacles have fused into an unpaired tentacle field. Beneath this field a cyclopic eye is situated, which is well-differentiated and shows indications of being double. In this respect, this case forms a transition to the synophthalmic embryos described below.

Limnaea KL 2-I. A small egg-mass, containing 29 eggs, was put in 0.001% LiCl immediately after oviposition; in the controls the formation of the 1st polar body began after 85 minutes. After 48 hours, the eggs were transferred to tap water at the gastrula stage. On the 3rd day, the gastrulae proved to have an abnormally opaque appearance. On the 10th day, they had developed into 6 nearly normal young snails, 1 synophthalmic, 1 triophthalmic, 3 cyclopic embryos, 1 monophthalmic with right eye missing, 3 anophthalmic-acephalic and 5 teratomorphic embryos; 9 embryos had died. The cyclopic embryos were fixed on the 13th day in Bouin.

KL 2-I was sectioned in a sagittal direction, beginning from the right side. The first sections through the head pass through the right cerebral, pedal and pleural ganglia and the supra-intestinal and abdominal ganglia; furthermore, the right oral

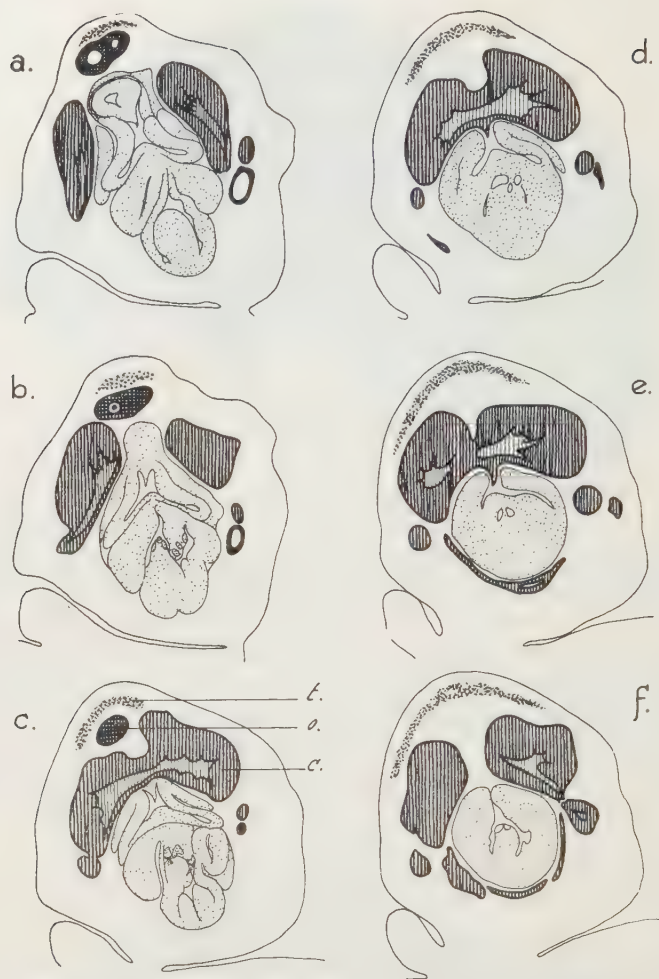


Fig. 12. *Limnaea OW 3-II*. Series of transverse sections through the head. *c.* unpaired cerebral ganglion. *o.* cyclopic eye. *t.* unpaired tentacle field.

tentacle and lateral tentacle are touched (fig. 13a). 5 Sections further, the right pedal ganglion passes into the pedal commissure (fig. 13b); the right buccal ganglion is situated against the posterior side of the buccal mass. After another 5 sections, the mouth is touched (fig. 13c). On the dorsal side, it is bordered by the anterior part of the apical plate; this consists of 3 cells, which are ciliated but not flattened as in normal embryos (fig. 14). Behind



Fig. 13. *Limnaea* KL 2-I. Sagittal sections through the head. a. abdominal ganglion. a.p. apical plate. b. buccal ganglion. c. cerebral g. c.c. cerebral commissure. l.t. lateral tentacle. m. mouth. o.t. oral tentacle. p. pedal ganglion. par. parietal ganglion. p.c. pedal commissure. pl. pleural ganglion. s.i. supraintestinal g. st. statocyst.

these cells, the dorsal part of the head is covered by a tentacle field which extends from one side to the other across the head. Beneath the tentacle field, a large single eye is situated; it is well-differentiated with pigment layer and a big lens. Posteriorly to the eye, the median angle of the right cerebral ganglion is still



Fig. 14. *Limnaea* KL 2-I. Sagittal section through the head, cf. fig. 13c.
a.p. apical plate. c. cerebral ganglion. e. eye. t. tentacle field.

visible. 2 Sections further (fig. 13d), this passes into the cerebral commissure, consisting of a fiber tract not surrounded by ganglion cells. In the next section, however, it is already submerged in the ganglion cell mass of the left cerebral ganglion. Finally, the section of fig. 13e shows the left cerebral and pedal ganglia and the parietal ganglion, furthermore the left statocyst.

Summarizing, in this case the cerebral ganglia have not fused; there is a short but distinct cerebral commissure. The anterior

part of the apical plate is present, though somewhat abnormal; the posterior part of the apical plate, the head vesicle and velum are lacking. There is an unpaired tentacle field. The cyclopic eye is situated beneath this field and in front of the cerebral commissure; it is well-differentiated.

Altogether, 15 cyclopic embryos have been studied. In all, the nervous system is complete; the 11 ganglia can all be distinguished, with the exception of 2 embryos which are not yet old enough and where part of the ganglia are not yet clearly delimited. However, in many cases the cerebral commissure is abnormally short, often entirely embedded in the mass of the cerebral ganglia which have fused in the median plane. This condition exists in 9 of the 15 embryos, whereas in the remaining 6 a distinct cerebral commissure, not surrounded by ganglion cells, is present.

In all cyclopic embryos, the posterior part of the apical plate is lacking; on the contrary, the anterior part, consisting of 2-4 cells, is present in 11 of the 15 embryos though often somewhat abnormal in its differentiation; in the remaining 4 it is lacking, too. Head vesicle cells are present in 9 cases, absent in 5, uncertain in 1; a velum is present in 6, absent in 7, uncertain in 2 cases.

The rudiments of the lateral tentacles have fused into a single tentacle field in 10 cases; they are separated in 3 embryos, whereas in 2 cases this could not be made out with certainty. No correlation can be ascertained between the shortening of the cerebral commissure, the absence of the anterior part of the apical plate and the fusion of the tentacle rudiments; on the contrary, a strong correlation exists between the head vesicle and velum.

SYNOPHTHALMIC EMBRYOS

Limnaea JT 7-IV: An egg-mass, consisting of 79 eggs, was put in 0.01% LiCl solution 30 minutes before the formation of the 1st polar body in the controls; it was transferred to tap water after 1 hour and washed thoroughly. The egg mass gave rise to 27 nearly normal young snails, 15 synophthalmic, 8 triophthalmic, 8 cyclopic, 4 anophthalmic, 1 acephalic and 4 teratomorphic embryos; 12 embryos died before the type of malformation could be determined. 9 Synophthalmic embryos were fixed on the 15th day (Plate XVIII, fig. 3).

JT 7-IV was sectioned transversely. Two well-developed eyes are situated in the dorsal part of the head near the median plane (plate XVIII fig. 4); they are nearly in touch, but not fused with one another. Immediately behind the eyes an unpaired median tentacle field begins (plate XIX, fig. 1), which extends backwards over 9 sections at $7\frac{1}{2}\mu$. On the other hand, the oral tentacles, on either side of the mouth, are paired (plate XVIII, fig. 4; plate XIX, fig. 1) and separated widely from the mediodorsal tentacle field. The apical plate is entirely lacking; the same holds true of the head vesicle and velum. The nervous system is intact, but the cerebral ganglia have fused dorsally to the pharynx; the cerebral commissure, though well-developed, is nearly entirely covered by ganglion cells on its anterior and dorsal face (plate XIX, fig. 2).

Altogether, 15 synophthalmic embryos have been studied. In all, the nervous system is complete, with the exception of a shortening of the cerebral commissure which is evident in 8 of the 15 cases.

In all synophthalmic embryos studied, the posterior part of the apical plate is lacking; the anterior part is present in 12, absent in 3 cases. Head vesicle cells are present in 5, absent in 9 cases, uncertain in 1; velum is present in 4, absent in 10, uncertain in 1. The rudiments of the lateral tentacle field have fused in 12 cases; they are separated in 2 cases, uncertainty as to this existed in 1 case.

TRIOPHTHALMIC EMBRYOS

Limnaea JT 6-I. The embryo belongs to the same batch as JT 7-IV (p. 341). It was fixed on the 11th day and sectioned transversely.

Plate XIX, fig. 3 shows a section through the 3 eyes. They are situated near the median plane of the head, 2 to the left and somewhat more dorsally, one more ventrally and to the right. All are well-developed, with a distinct pigment layer and a lens; they are in contact with their outer surfaces, the 2 dorsal eyes somewhat flattened against each other, but not fused. On the ventral side of the head a ciliated groove is seen, forming the anterior part of the apical plate.

The study of the sections shows that the posterior part of the apical plate is lacking. So is the head vesicle, but a distinct velum is present on either side of the head. The lateral tentacles have

fused into a single median tentacle field. The nervous system is intact, but the cerebral ganglia have fused into a single unpaired mass which surrounds the short cerebral commissure.

4 Triophthalmic embryos have been studied. The posterior part of the apical plate is lacking in all cases; the anterior part is present in 2, lacking in 2 cases. The head vesicle is present in 1, lacking in 2, uncertain in 1 case; velum present in 2, lacking in 1, uncertain in 1.

In 3 cases the cerebral commissure is shortened and the tentacles have fused into a median tentacle field; in 1 case, uncertainty exists in both respects.

ANOPHTHALMIC EMBRYOS

4 Anophthalmic embryos have been studied; 2 of them belonged to batch JQ (cf. above p. 332, embryo JQ 2) and had been fixed on the 10th day, the other 2 belonged to batch JT (cf. embryo JT 7-IV, p. 341) and had been fixed on the 15th day.

In 3 of these embryos, the eyes are missing altogether; in 1, some pigmented cells are lying on either side against the cerebral ganglia, probably representing rudimentary eyes. The nervous system is intact, but the cerebral commissure is shortened and surrounded by ganglion cells in 3 of these embryos. The posterior part of the apical plate is lacking in all, the anterior part lacking in 2, present in 2 cases. The head vesicle is lacking in 3 cases, uncertain in 1, the velum lacking in 3, present in 1. The tentacle rudiments are separated in 1, fused in 1 and uncertain in 2 cases.

ACEPHALIC EMBRYOS

4 Acephalic embryos have been studied; 3 of them belonging to batch JQ (cf. above p. 332) and fixed on the 10th day; the fourth belongs to another batch, which had been treated with 0.005% LiCl for 6 hours, beginning immediately after laying. In all these embryos, the eyes are lacking. The posterior part of the apical plate is lacking in all, the anterior part lacking in 2 cases, present in the other 2. Both the head vesicle and velum are lacking in 2 cases, present in 2. The tentacle rudiments are fused in 3 of these embryos; in 1 case this could not be proved with certainty.

Besides these deficiencies, which are characteristic of the whole

series of malformations described above, in this group further reductions of the head organs occur. In 2 of these acephalic embryos, the left cerebral ganglion is lacking; in one of them, the left statocyst is missing, too. In a third embryo, the whole left half of the nervous system, except the pedal ganglion, is absent; the right cerebral ganglion is reduced, the left statocyst smaller than the right one. In the fourth embryo of this group both cerebral ganglia are greatly reduced in size and fused dorsally to the mouth cavity. Furthermore, in one embryo the pharynx is greatly reduced, the radular sac and a tubular oesophagus arising separately at the mouth.

DISCUSSION

In the 42 embryos, belonging to the group of cyclopic malformations, studied in this paper, besides the displacement, reduplication, fusion or reduction of the eyes, the following deviations of the normal structure of the head have been obtained with a certain degree of constancy: a shortening of the cerebral commissure, with fusion of the right and left cerebral ganglion in the median plane of the head; suppression of the anterior and posterior part of the apical plate, of the head vesicle and velum; and fusion of the lateral tentacle fields into a single median tentacle field. Only in the acephalic embryos, further reductions of head organs (nervous system, statocysts, pharynx) occur.

Of these malformations, the reduction of the posterior part of the apical plate is the most constant one; in fact, it has occurred in all of the 42 embryos studied. This reduction is, therefore, a characteristic trait of the embryos of the cyclopic series. It is followed, in order of incidence, by the fusion of the tentacle fields, occurring in 83% of certain cases; the shortening of the cerebral commissure (67%); the suppression of velum and head vesicle (61% and 55%, respectively) and of the anterior part of the apical plate (31%).

It is a remarkable fact that these diverse abnormalities are distributed at random over the various cases. Only between the suppression of the head vesicle and the velum a clear correlation exists (the contingency $\chi^2 = 22.589$, which has a probability < 0.001).

It is evident from this survey that the malformations of the ectodermal organs of the head are not attended, as a rule, with

defects in the mesodermal or entodermal organs. It is not possible, therefore, to explain the Li-effect by its primary action on a mesodermal substrate which, by altering the inductive power of the latter, would bring about secondarily the ectodermal malformations, as in Amphibia. My results do not lend support to the hypothesis that interactions comparable to the "embryonic induction" known in other groups play a part in the development of the head in *Limnaea*. It is necessary, therefore, to look for another explanation of the Li-effects.

In order to appreciate the above-mentioned results, one has to keep in mind the course of normal development, as it has been described above (p. oo). It is evident from fig. 1, that the region of the head between the eyes, the tentacles and the cerebral ganglia of both sides is formed by the cells in the neighbourhood of the animal pole.

We can arrive at a still more accurate statement by turning our attention to that structure the differentiation of which is suppressed in all our synophthalmic, triophthalmic, cyclopic, anophthalmic and acephalic embryos: the posterior part of the apical plate. Evidently, this structure forms the crux of our understanding of this group of malformations; if we can arrive at an explanation of its suppression in these embryos, we may hope to find the clue for a better understanding of the Li-effect.

It is of much interest to know the origin of the cells forming the posterior part of the apical plate in normal embryos. Unfortunately, the cell-lineage of *Limnaea* has not yet been studied in detail. However, in the nearly related forms *Planorbis* and *Physa* cell-lineage studies have been made by HOLMES (1900) and WIERZEJSKI (1905). In both forms, the derivation of the above-mentioned cells is quite the same. In view of the close correspondence in development between the fresh-water pulmonates studied so far, it seems justified to extend these results to *Limnaea* also. Until further notice, it is assumed, therefore, that the cell-lineage in *Limnaea* corresponds to that found in *Planorbis* and *Physa*.

According to HOLMES and WIERZEJSKI, the posterior 4 cells of the apical plate are the cells 1a^{III}, 1b^{III}, 1c^{III}, and 1d^{III}, i.e. the apical cells, which surround the original animal pole of the egg. The treatment of the eggs with lithium has, therefore, caused the suppression of the differentiation of the cells situated at the animal pole.

In the various cases, this suppressing influence may have extended more or less far around its center at the animal pole. Accordingly, structures derived from adjacent cells may be affected more or less, too. If, for instance, besides the cells 1^{11} also their sister cells 1^{12} belong to the affected region, the anterior part of the apical plate and medial parts of the tentacle fields will be inhibited; the latter effect will cause a further medial displacement of the eyes and the cerebral ganglia. With further extension of the effect to the cells 1^{12} and 1^{21} the head vesicle and velum will be affected, too. Slight irregularities in the extension of the inhibiting influence in different directions may account for the random character of the combination of effects in the various cases. The fact that the velum and head vesicle are suppressed somewhat more often, the anterior part of the apical plate less often than might be expected, may perhaps indicate that the Li-effect extends more easily in a posterior direction than anteriorly.

It should be emphasized once more that the Li-effect is not due to a local cell necrosis; the development of these malformations is not attended with a loss of cells. Hence, it must be concluded that the action of Li has brought about a change in the pattern of determination of the head organs and, therefore, in the differentiation of the cells. The eyes of the cyclopic embryos develop from cells which in normal development would have given rise to median structures e.g. apical plate.

The observation that the effects of Li treatment are most pronounced at the animal pole and diminish in intensity with increasing distance from this pole is of great importance. They prove that in *Limnaea* lithium acts on a gradient-field as it does in the Echinoids and the Amphibia (cf. DALCQ 1941, p. 00). In all these cases, it has a depressing influence on the maximum of the gradient field; in sea urchins and *Limnaea*, this maximum is situated at the animal pole, whereas it is found in the Amphibia in the middle of the dorsal blastopore lip. By virtue of this depressing action, the cells in this region are forced to differentiate in a way characteristic of lower levels of the gradient in normal embryos: somites in stead of notochord in the Amphibia (LEHMANN 1937), tentacle field and eye in stead of apical plate in *Limnaea*.

It is evident, therefore, that a polar gradient field plays an important part in the development of *Limnaea*. As a matter of course, this is not the only factor; it does not suffice to explain

the pattern of determination in normal development. A polar gradient field could in itself only explain a concentric arrangement of organs around the animal pole. Other factors must be present to modify this simple pattern. Nevertheless, one of the important components of the determination process in *Limnaea* has been detected herewith.

It is a remarkable fact that in many cases we have good evidence of the operation of gradient-fields in development (RAVEN 1943) whereas we know next to nothing about their nature and manner of action. In order to get some information as to this, we must proceed along two different paths. First, we must try to catalogue the processes of normal development which might be ascribed to the action of the presumed gradient-field, and deduce from them some common characteristics. Secondly, the study of the direct effects of agents which are known to have an influence upon the conformation of the gradient-field may give us some clue as to its nature.

In a previous paper (RAVEN 1946, p. 420), I have listed the processes of normal development of *Limnaea* which might be ascribed to the action of a polar gradient-field. In summary, it may be said that the phenomena of normal development suggest that the animal-vegetative gradient-field of the egg acts 1°. on the nuclear and mitotic apparatus, determining its position in the neighbourhood of the animal pole during maturation and the first 3 cleavage cycles, and cooperating in the determination of the direction of the spindles in these and later divisions; 2°. on the subcortical plasm, causing its accumulation at the vegetative pole prior to oviposition, and its attraction to the animal side immediately before the 3rd cleavage; 3°. on the animal pole plasm, attracting it to the animal side in the last hour before the 1st cleavage; 4°. on the ecto- and endoplasm, determining their differential distribution during the later phases of cleavage.

Here, some further details will be enumerated which characterize the cells in the immediate neighbourhood of the animal pole. At the gastrula stage, a depression is formed at the animal pole; here a few cells are found, which are distinguished by the presence of extremely big albumen vacuoles. Mostly, they contain one vacuole, the diameter of which largely surpasses that of the nucleus, and a number of smaller ones. The contents of these big vacuoles are not homogeneous, but consist of several heavily staining rods lying irregularly dispersed. The cells surrounding

the polar depression contain somewhat more glycogen than the other cells of the animal hemisphere.

In the beginning of differentiation, the cells of the apical plate are prismatic or conical in shape, and contain numerous rather big albumen vacuoles, the contents of which consist of irregular morsels and rod-like pieces of coagulum. These cells and the anterior cells of the head vesicle are most heavily stained by neutral red, Nile blue hydrochloride and brilliant cresyl violet; in the albumen vacuoles, the pseudocrystals of albumen can be seen in such eggs in the living state.

Recently, we have made some attempts to attack the problem of the Li-action from the other side mentioned above. The analysis of a great number of experiments showed that this action exhibits a clearly marked phase specificity (RAVEN, KLOEK, KUIPER and DE JONG 1947). Treatment of the eggs at the moment of 2nd cleavage gives about 100% exogastrulae; there is a distinct maximum of sensibility of the egg at this moment. On the other hand, there are two separate maxima of sensibility for the production of head malformations: one immediately after oviposition, a second at a rather advanced cleavage stage. Recent experiments (RAVEN and RIJVEN 1948) have shown that this second maximum is reached 2-3 hours after the beginning of the 24-cell stage; there is a gradual rise in sensibility until this moment and a relatively sudden drop thereafter.

DE VRIES (1947) studied the influence of LiCl solutions on viscosity and tension at the surface of uncleaved *Limnaea* eggs. 0.1-1% LiCl solutions caused a decrease of viscosity, whereas 0.006-0.03% solutions produced a slight but significant increase. The tension at the surface was increased by 0.6-1% solutions, decreased by 0.1% solutions. It may be concluded that LiCl in the concentrations used for the production of head malformations increases the viscosity of the eggs but has no effect on the tension at the surface strong enough to be detected by the centrifugation method employed.

In eggs treated with 0.001-0.01% LiCl solutions, no direct effect of the treatment on their structure and early development can be observed. Only later on deviations from the normal course of development occur, leading either to exogastrulation or to the production of head malformations.

In order to get some insight into the direct action of LiCl solutions on the egg, DE GROOT (1948) studied the influence

of higher concentrations of this salt on eggs immediately after oviposition. In general, development was blocked the earlier, the higher the concentration used. With 1-0.4% solutions, which are hypertonic to the eggs, especially the evolution of the nuclear and spindle apparatus is affected; depending on concentration, it may be blocked at different moments: before or immediately after the extrusion of the 1st polar body, at the stage of 2nd maturation spindle, after the extrusion of the 2nd polar body or still later, after the copulation of the pronuclei. Often, a depolarization of the eggs has taken place: the 2nd maturation spindle sinks into the egg and places itself perpendicularly to the egg axis; or, after the formation of both polar bodies, the karyomeres formed by the egg chromosomes do not remain at the animal pole, but are shifted to a deeper position. In the latter case, the migration of the sperm nucleus is delayed; in other cases, however, where the normal course of maturation is deflected immediately after the extrusion of the 1st polar body and karyomeres are formed prematurely by the egg chromosomes, the formation and migration of the male pronucleus may be accelerated by $1-1\frac{1}{2}$ hours.

Probably, most of these disturbances of the mitotic cycle of the egg may be ascribed to the hypertonicity of the medium. This is indicated by the fact that similar phenomena have been observed in eggs treated with a hypertonic CaCl_2 -solution (RAVEN and MIGHORST, 1946), whilst a suppression of maturation and cleavage can also be brought about by treatment with urea and sucrose solutions of similar osmotic pressures (RAVEN and KLOMP 1946).

On the other hand, in 0.2% LiCl solutions, which are nearly isotonic to the eggs, the evolution of the nuclear and spindle apparatus proceeds normally. However, here a disturbance of cytoplasmic movements becomes very evident. The subcortical plasm shows a very abnormal distribution, great accumulations of this plasm occurring at various places. Furthermore, the formation of the animal pole plasm is altogether suppressed in all concentrations studied (0.2-1.0%). It is probable that this disturbance of cytoplasmic movements is a specific lithium effect; in a 0.75% thiourea solution, which is likewise about isotonic to the eggs, a distinct formation of the animal pole plasm occurs (SOBELS, 1948).

Summarizing these experiments, it may be said that the polar gradient field of the egg may be affected in two ways: 1°. by

hypertonic solutions, leading to disturbances in the nuclear and spindle apparatus, often having the character of depolarization phenomena, 2°. by a specific lithium influence, disturbing the normal course of cytoplasmic movements. This points to a difference in the nature of the factors directing both groups of phenomena: the evolution of the mitotic apparatus, on one hand, the cytoplasmic movements, on the other. Further investigations will have to elucidate the true nature of both groups of factors.

The observation that the distribution of cytoplasmic substances in the egg is disturbed, in a specific way, by lithium and that, especially, the local accumulation of the animal pole plasm is suppressed, is, in my opinion, of the utmost importance. It is true, the concentrations employed in these experiments exceed many times those used for the production of exogastrulation or head malformations. It is conceivable, however, that the same salt in greater dilutions may lead to minor disturbances of the same nature, not clearly observable with our ordinary methods, or only taking place at a somewhat later stage of development. In this way, the chemodifferentiation along the egg axis would be hampered, and the "chemical gradient-field" through the egg would be levelled. It is clear that such a phenomenon would explain the suppression of apical differentiations, such as has been found in the embryos described in this paper.

SUMMARY

1. The structure of the head in 42 cyclopic, synophthalmic, triophthalmic, anophthalmic and acephalic embryos of *Limnaea stagnalis*, obtained by the action of lithium, has been studied.

2. Besides the displacement, reduplication, fusion or reduction of the eyes, the following deviations of the normal structure of the head have been obtained: a shortening of the cerebral commissure, with fusion of the right and left cerebral ganglion in the median plane of the head; suppression of the anterior and posterior part of the apical plate, of the head vesicle and velum; and fusion of the lateral tentacle fields into a single median tentacle field. Only in the acephalic embryos, further reductions of head organs (nervous system, statocysts, pharynx) occur.

3. The suppression of the differentiation of the 4 posterior cells of the apical plate occurs with absolute constancy in these embryos. In normal development, these cells are derived from

the apical cells, which surround the animal pole of the egg.

4. The results do not lend support to the hypothesis that interactions comparable to the "embryonic induction" known in other groups play a part in the development of the head of *Limnaea*.

5. They prove, however, that lithium acts on a gradient-field in *Limnaea* as it does in the echinoids and the amphibia.

6. Recent experiments on the direct effects of lithium on the eggs of *Limnaea* are discussed.

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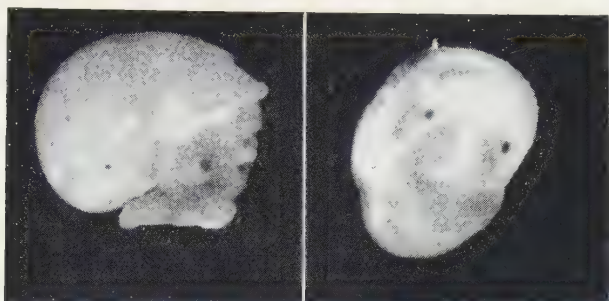


Fig. 1. "Hippo-stage" embryo; *a.* from the right side, *b.* from the anterior side.

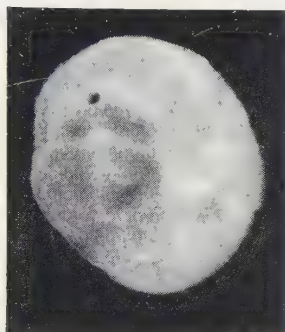


Fig. 2. Cyclopic embryo from lithium-culture (JQ).

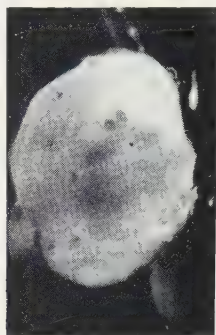


Fig. 3. Synophthalmic embryo from lithium culture (JT).



Fig. 4. *Linnaea* JT 7-IV. Transverse section through the head. Photo, 150 x. Synophthalmic eyes.

Fig. 1. *Limnaea*
JT 7-IV. Trans-
verse section
through the head.
Photo, 150 x.
Unpaired dorsal
tentacle field.

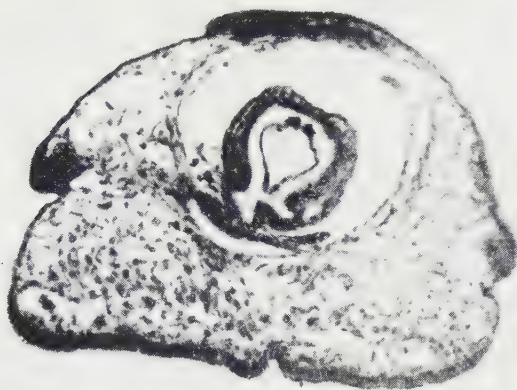


Fig. 2. *Limnaea*
JT 7-IV. Trans-
verse section
through the head.
Photo, 150 x.
Fused cerebral
ganglia (c.).

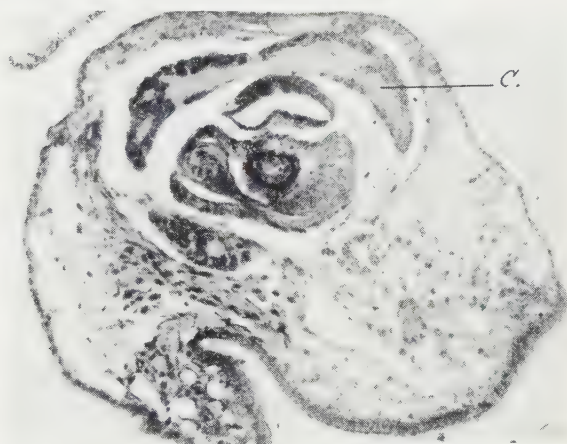


Fig. 3. *Limnaea JT 6-I*. Tangential
section through mantle fold, head
and foot. 3 Eyes, anterior part of
apical plate (a.p.).

THE TOOTHPLATE-FORAMINIFERA

BY

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A large group of Foraminifera has been gathered in a new Ordo of that class of Protozoa, the Dentata.

These Dentata show as a peculiar inner structure a more or less developed, often contorted plate, running from a former, now septal foramen to the next one, through a chamber. This plate (fig. 1) often is attached with one of its sides to the axial wall of a chamber, and shows an often folded free border on the opposite side, which in many cases is ornamentated by typical denticulations or other structures (fig. 2). Deriving from these more primitive forms are groups, in which the toothplate is more or less reduced, or gives rise to the building of peculiar canalsystems.

All members of these Dentata show pores in the walls, even those of the basal group, the Valvulinidae. This fact leads to the conclusion, that the difference between agglutinantia and calcareine forms is not so large, as hitherto has been supposed, for the Valvulinidae have been regarded as agglutinantia (fig. 3).

The foramen, towards which the toothplate is running, always is attached to this toothplate with one of its borders, the proximal border in respect to the proloculus, and often the plate forms also a larger or smaller part of the foraminal area (fig. 4). Such foramina are called here protoforamina. A large group of Dentata show but protoforamina and have been gathered in the sub-orde of the Protoforaminata. To this sub-orde are belonging the families Bolivinidae, Cassidulinidae, Buliminellidae, Buliminidae, Virgulinidae, Uvigerinidae and the basal-family of the Dentata, the Valvulinidae.

As the toothplate is formed together with the newly built chamber, and within it thus totally surrounded by protoplasm from the beginning it is not exposed to different outer circumstances, and forms in this way a very important, for the species very constant, character. Many species, hitherto uncertain of systematic place, could be unraveled in respect to their taxonomy with the aid of the study of their toothplates. It was shown furthermore, that within a family or a genus, the study of the

toothplates reveals not only relationships between the species of the family or genus, but also forms a very clear indicator as to the development of the family and the genus. This development, based on the toothplates, was in striking correspondance with the geological age of the different species studied.

In the Protoforaminata the more primitive form of toothplate shows a folded plate, attached to the axial or basal chamberwall, and running from the former protoforamen toward the next one, always from the distal border of the former protoforamen toward the proximal one of the next foramen (fig. 1). Its free folded part forms at the inner side of the next foramen a more or less pronounced tooth, which often forms peculiar structures or a protruding tongue. This is the case in the forms, in which the chambers are spirally arranged along an axis of the test, which spiral shows in most primitive forms five or four chambers in a whorl (Buliminellidae), which in the Buliminidae is reduced to three, as in the Uvigerinidae, whereas in the Virgulinidae and the Bolivinidae a whorl has at the oral end even two chambers. In many forms, however, the arrangement of the chambers becomes at the end uniserial, and in these cases the toothplate always loosens its attached part from the chamber wall, thus becoming more or less symmetrical and running straight through the chamber to the next foramen. But, when such an uniserial form is deriving from triserial *Bulimina* or *Uvigerina*, the angle between successive toothplates maintains 120° , whereas when it is deriving from *Bolivina*, its angle constantly is 180° . Here too one observed a rule which gives us the possibility to classify these aberrant forms. But the attachment of the toothplates always to the opposite border of the next foramen in respect to the border, to which its base is attached to the former foramen, is also maintained (fig. 5).

A very peculiar fact was discovered when studying the pores of the Dentata. All primitive forms, deriving from jurassic and cretaceous sources, as well as the primitive descendants in later times, show fine pores in their walls. Several groups maintain these fine pores, which have been indicated as protopores, in all their species, viz. the Buliminellidae, the Virgulinidae and the Uvigerinidae. Later developed or more advanced species show, on the contrary, often larger pores, or the pores are more scattered on the surface of the test. In some forms, f.i. paleocene *Bulimina trigonalis* Ten Dam, the initial chambers show the fine primitive pores, whereas the later formed chambers show larger

pores. In other groups of *Dentata*, f.i. in the genera *Cibicides* and *Siphonina*, the protopores are arranged in small groups, which often fuse into a single larger porus, a deuteropore (fig. 6). Deuteropores are always found up in more highly specialised groups, and in the genus *Gavelinella* it could be shown, that more primitive and geologically older forms show fine protopores, whereas later and more developed forms show deuteropores (fig. 7). Thus a tendency in the whole group of the *Dentata* was found to develop larger pores in more advanced groups. This character, the situation and size of the pores in the walls showed to be a very constant one in the different species, and in the Globigerinidae it could be used to enravel this difficult genus into many constant species by means of the "pore-index" (a combination of size of pores and number of pores occurring in a surface-unit; fig. 8).

In a second group of *Dentata* the spiral of test consists of five or more chambers in a whorl, so that typical conical and coiled forms arise. In more primitive forms of this second group or sub-ordo the walls show fine protopores and are more or less opaque of structure, bearing a yellowish or brownish colour. Each chamber shows a single foramen at its distal wall, and the toothplate is running as in the Protoforaminata. But in many higher forms a second foramen is developed next to the normal protoforamen with its toothplate, not connected with the plate, and always situated more axially in respect to the protoforamen, and more distally (fig. 9). In most cases this deuteroforamen, as it has been called here, is situated quite at the ventral suture of the last-formed chamber. So the whole group has been called Biforaminata, in respect to these two foramina at the outer wall of the last-formed chamber. A basal genus of typical biforamate structure is *Epistomina*, with a large protoforamen just ventrally to the margin of the coiled test, and a deuteroforamen at the ventral suture of the chamber formed when a new chamber is added. A large and well-developed toothplate is found up in the chambers in more primitive forms of geologically older date (jurassic and cretaceous), whereas in younger and more developed species of this genus the toothplate is found only in the last-formed chamber, in older chambers it being dissolved but for some ridges, and the protoforamen is closed in these chambers by secondary chalk. From those forms, which in this sub-ordo of the Biforaminata show yet a single protoforamen (the more primi-

tive forms such as *Lamarckina* and *Ceratobulimina*) have developed several very important groups, in which the toothplate is more or less obliterated. A reduced toothplate is already found in *Pulvinulinella*, and in its descendant, *Eponides*, it is found only in minor traces; in *Parrella* it is found in a reduced state, and in descended genera, such as *Siphonina* and in *Cibicides*, often only the place of the single foramen indicates its real homology with a protoforamen. This place always is proximal and marginal. I could trace many primitive species in these genera, which undoubtedly show a line of development in the direction of reduction of the toothplate together with enlarging of the pores (fig. 10).

A very important group has obviously developed from the typical forms with two foramina in the distal wall of each chamber, the typical biforaminate forms, with the basal genus *Epistomina*. In this group the toothplate is developed into a very complicated structure, giving rise to canals and accessorial foramina next to the two foramina, already mentioned. To these highly developed genera belong the genera *Robertinoides* (from which the inner structure has been cleared up already by HÖGLUND in 1947, though he did not homologise the inner structures of the chambers with the toothplate of the Protoforaminata), *Pseudobulimina* and *Cushmanella*. In the latter genus the toothplate reaches its highest development of the Dentata, giving rise to a symmetrically built test with two accessorial openings near to the deuteroforamen (fig. 11). I could show, that from this toothplate of *Cushmanella* (closely allied genera are already known in the Upper Cretaceous and early Eocene) have developed the canal-systems of the families Polystomellidae and Camerinidae, thus proving, that these two closely allied families have derived without doubt from Biforaminate Foraminifera (fig. 12), whereas the Nonionidae have developed from the genus *Pseudobulimina*, while many forms, hitherto known as Polystomellidae (*Elphidium*) in reality belong to the Nonionidae when considering the finer structure of their canal-systems. For these latter forms, gathered into the new genus *Elphidiononion* (genotype: *Elphidiononion poeyanum* (d'Orbigny)), show nothing of the typical canalsystem of real *Elphidium*, and never show real retral processes of the chambers, but solid pillars in their place (fig. 13).

A third group among the Biforaminata has reduced the protoforamen more or less, while the deuteroforamen is developed and remains in extremely developed forms the only observable one.

The place of this deuteroforamen however distal in respect to the initial part and ventral in respect to the margin, sutural in respect to the suture at the ventral side, indicates its real status (fig. 14). In this group, intermediate forms between fully developed protoforamen and totally reduced ones are found in the genus *Alabamina* (fig. 15), from which several other forms with only a deuteroforamen have developed, f.i. *Gyroidina*. A peculiar genus forms *Laticarinina*, in which the deuteroforamen is developed, the protoforamen is closed, but the toothplate is very highly developed forming not only a partial septum in each chamber, but also a broad carina at the margin of the test.

In the third group of Foraminifera, belonging to the Dentata, the sub-ordo of the Deuteroforaminata, the tendency to preserve only the deuteroforamen, whereas the protoforamen is reduced, is prominent.

The basal genus of this group undoubtedly is jurassic *Conorbis* (genotype: *Conorbis mitra* Hofker from the Albien); it is characterised by opaque, yellowish walls, as is the case in neighbouring *Lamarkina*, and only a single foramen is traceable in the last-formed chamber, the apertural face of which is found at the flattened or concave base of the typical conical tests. Here too the walls show fine protopores, as is the case in all primitive species, and the spiral of chambers shows four or five chambers in a whorl which, however, in later whorls may be reduced to three or even two. Typical for the group in primitive forms is the shape of the toothplate, a vertically placed pillar running from the former protoforamen to the axial border of the next one (fig. 16). From this genus *Conorbis* have derived without any doubt those forms, with conical test, and opaque walls, called by BROTZEN (1948) *Rosalina*, but, as several other forms have been mentioned likewise with this name by d'ORBIGNY, I prefer to call them *Neoconorbina* (genotype: *Neoconorbina orbicularis* (Terquem)). In that genus the protoforamen begins to obliterate, and has been designed by BROTZEN as lobus posterioris, and a second foramen has been developed, called by BROTZEN lobus anterioris. This deuteroforamen is separated from the protoforamen by a peculiar lip, called by BROTZEN tenon (fig. 17). This lip has been formed by the toothplate, which in this genus likewise is reduced, but always visible, and pillar-shaped. In another direction has developed the group, hitherto known as *Patellina*, *Patellinoides*, etc. Here the toothplate is fully developed, and a second

foramen has not yet appeared. I could state, that all species, known as *Patellina* and *Patellinoides* do not show in the initial part (as has been hitherto believed) a spiral without septa (which has given rise to the opinion, that the genus *Patellina* was a descendant from *Spirillina*), but that in the contrary all genera and species observed show a more or less highly developed conorbine initial part, with fine, only in a clarifier visible, septa (fig. 18). The toothplate is that of *Conorbis*, but for the fact, that in higher developed *Patellina* this toothplate gives rise to secondary septa in the chambers, which form partial chamberlets. All forms show highly hyaline walls, and the pores are often more or less absent. As the typical feature of the group is the reduction of the number of chambers in a whorl up to two, I propose a single genus for the whole group, as the differences between the genera are more special than generic, *Discobolivina* (genotype: *Discobolivina corrugata* (Williamson)).

A very peculiar group has arisen from *Conorbis* by forming a second foramen and closing the protoforamen by a special plate, pierced with fine protopores. This group has been called the family of the Amphisteginidae. There is a jurassic form for instance, *Reinholdella dreheri*, which forms the link between *Conorbis* and *Asterigerina*, and in which the toothplate shows the more vertical placing of *Conorbis*, but in which the hollow of the protoforamen (lobus posterioris) is closed by this porous plate, giving rise to partial secondary chamberlets at the ventral side of the test, alternating and intercalated between the normal chambers (fig. 19). This process is more highly advanced in *Asterigerina*, in which the toothplate is converted into a more horizontally placed septum between the normal chamber and the secondary one, but always showing its typical structure of toothplate, running from the former protoforamen towards the next one (fig. 20). The protoforamen is the opening between the normal chamber and the secondary ventral one, and the deuteroforamen is the foramen seen at the apertural face of the last-formed chamber. From *Asterigerina* has developed the genus *Amphistegina*, by overlapping of the chambers at the dorsal side and by reducing the bulk of the secondary chambers, at the ventral side. Hitherto it was believed by BROTZEN and myself, that *Amphistegina* had nothing to do with *Asterigerina*, but the study of young specimens of *Amphistegina* has shown its clear relation with *Asterigerina* by discovering the toothplate dividing the secondary cham-

bers at the ventral side of the test from the normal chambers.

A second group of forms has reduced the protoforamen by diminishing its size, up to total repression of its opening. In this case the toothplate likewise is reduced and at the end totally obliterated. To this group are belonging all those forms, hitherto known as *Discorbis* or *Discorbina*; I propose here a new name, as quite other species also have been called *Discorbis*, not belonging to this typical species group. I have given the genus the name of *Discopulvinulina* (genotype: *Discopulvinulina bertheloti* (d'Orbigny)), as the species formerly known as *Pulvinulina* seem likewise to belong to this new genus. At the dorsal side all chambers are visible in primitive species, but gradually they may in higher advanced forms overlap the dorsal side, and even may reach each other at the centre of the dorsal side (fig. 22). These forms show a very high resemblance with some species of *Cibicides*, but for the finer pores and the shape of the foramen, which is here a deutoforamen. For, at the ventral side of the species of this genus *Discopulvinulina*, the protoforamen is more or less totally reduced, whereas the lip and the deutoforamen are fully developed. Yet often the protoforamen can be found as a peculiar sharp groove behind the lip. The lip always is lacking pores, and thus shows its descent from the toothplate (fig. 22).

In some forms of *Discopulvinulina* the poreless lip is connected with a poreless part of the wall at the margin. It seems, that this poreless margin likewise has something to do with the original toothplate. These forms point to a next family, the Marginolamellidae, always with a poreless margin, and always with a lip behind the deutoforamen. To that family I brought the genera *Globotruncana*, *Globorotalia*, *Rotalipora*, *Cancris* (fig. 23).

In a second family, deriving from *Discopulvinulina*, the deutoforamen and the prominent lip are always present, but the margin is not poreless as in many typical species of *Discopulvinulina* itself. To that family, the Valvulinidae, have been gathered the genera *Valvulinella*, *Gavelinella*, the new genus *Gavelinopsis* (genotype: *Gavelinopsis atlantica* Hofker), and *Gyroidinoides*. In *Gavelinella* and *Gavelinellopsis* a typical character is the lacking of pores at the dorsal walls, which character is found also in *Gyroidinoides* (fig. 24). BROTZEN tried to prove, that the genera *Cibicides*, *Planulina* and *Anomalina* and some allied forms derived from *Gavelinella*; but in the Cibicidae the pores are mainly lacking at the ventral sides, and not at the dorsal ones, and in *Cibicides* the

foramen is a protoforamen, not a deutoforamen with the neoconorbine or discopulvinuline lip. So both groups are not at all related to one another (fig. 25).

From *Valvulineria* without doubt have developed the Globigerinidae, in which group I could establish the different species by means of the pore-index.

In quite a different direction from *Discopulvinulina* have developed the Cymbaloporetidae and the Tinoporidae. These important families have somewhat contorted their chambers, which is yet visible when comparing younger and older chambers of a test, and in such a way, as to maintain the protoforamen which forms a retral foramen of the chamber, by developing the deutoforamen in the same way at the anterior side of the chamber, while in the centre, towards the umbilical region, a third opening exists, formed by the protruding lip between proto- en deutoforamen (fig. 26). In this way three foramina have developed in *Cymbaloporetta*, of nearly equal size and equal function, while the lip remains and forms in some cases a bridge over the umbilical hollow. In the Tinoporidae this third opening is more or less reduced, but the two foramina, deriving from a proto- and a deutoforamen, remain.

In the Deutoforaminata once more the phenomenon was traced, that in geologically older or more primitive forms pores are fine protopores, whereas in higher advanced forms deuto-pores develop. This phenomenon was proved to exist in the genera *Discopulvinulina*, *Cavelinella* and in the Cymbaloporetidae and Tinoporidae, where only deuto-pores are found; in the genus *Globigerina* likewise primitive forms show finer pores.

Furthermore it could be stated, that a fourth group deriving from *Conorbis* is formed by the Rotalidae, in which group a new genus was discovered, *Asterorotalia* (Genotype: *Asterorotalia pulchella* (d'Orbigny)), with porous walls over the opening of the sutures and the protoforamen, enclosing a space, which opens at the ventral margin of the test (fig. 27). Several forms, hitherto called *Rotalia*, are belonging to this highly developed genus. In the Rotalidae too more primitive species and genera (*Streblus*) show fine protopores, whereas the groups, derived from that genus (*Calcarina* and *Baculogypsina*) show much coarser pores.

Much attention was given to the reproduction of the more than 400 species, which have been studied in two large publications, now in the press (Foraminifera from the Siboga-Expedition, part

III, *Dentata*; Foraminifera from the West-Indies, gathered by Dr. Mortensen's Pacific-Expedition). It could be shown, that statistical treatment of some characters of specimens of a species can be used for that purpose: the size of proloculus, size of the whole test, total number of chambers, and some other characters (fig. 30). When combined, these characters give a diagram, which enables us to conclude about the reproduction-scheme, followed by the species. MEYERS, LE CALVEZ and myself have pointed out, that in the Foraminifera, several different schemes of reproduction can be found. The more primitive form of reproduction is that, which leads to so-called trimorphism: a test with very small proloculus (size up to 10 μ diameter) is formed by fusing of gametes; these flagellospores always derive from individuals with much larger proloculus which, in some forms such as *Neoconorbina* and *Buliminella*, fuse at their mouths to enable the spores to fuse (fig. 29). This B- or micropheric generation gives rise to the forming of agametes, which give rise to a much larger proloculus, and hereafter several other generations may follow, ending with the production of very large agametes or plasmodiospores, which give rise to individuals with very large proloculus; these individuals form on their turn the microgametes (fig. 28).

A second form of reproduction is found, when one or more of the generations is repressed. Often only a single A-form (with large proloculus) is intercalated between two microspheric generations. In this case dimorphism arises. In some forms, however, even a single kind of generation is maintained, and, as I found, in that case often the microspheric generation is the remaining one. This generation seems to be totally apogamic. Alterations of these three schemes seem also to occur (fig. 29).

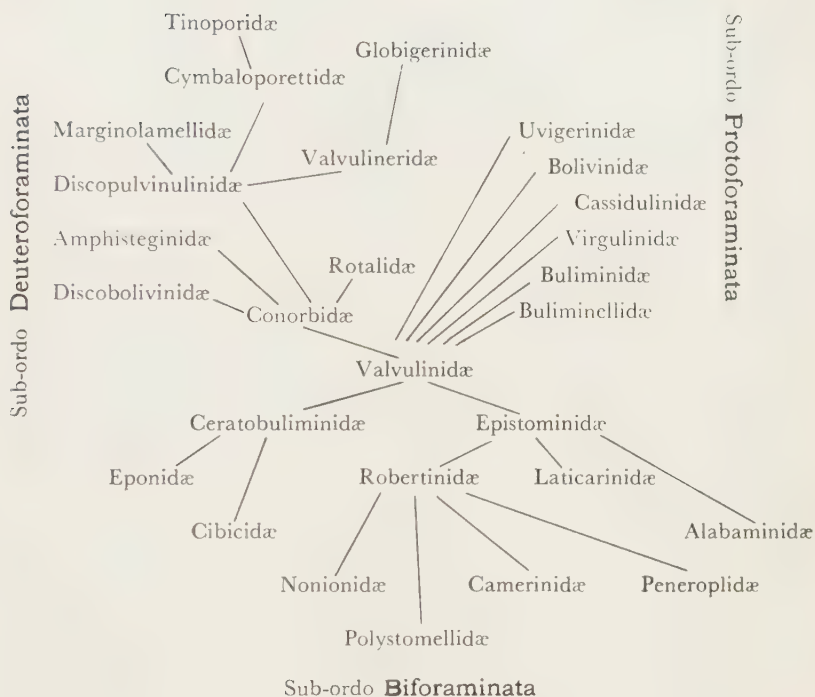
Now it was a remarkable fact that in more primitive forms or in geologically older forms the trimorphic reproduction-scheme is a rule, whereas in higher advanced or geologically younger forms the dimorphic scheme or even the apogamic form of reproduction often becomes the dominant one. This was very clearly shown in the Boliviniidae, the Conorbidae and the Globigerinidae. I could state it recently in the genus *Asterigerina* also. It was traced too in several groups of the Biforaminata. So it seems to be the rule, that the scheme of reproduction is simplified in time and in development of the Foraminifera. Pelagic forms more specially show to have the tendency to simplify the method of reproduction

up to apogamy, and here always the microspheric form is the remaining generation. May be, that in case of apogamy the sexual microgametes perform a kind of amphimixis, as has been found in *Paramaecium*, which amphimixis cannot be performed in plasmodiospores, which are asexual, the parent specimen having a plasmodium.

In the larger papers, mentioned above, I have tried to solve many very confusing problems in respect to taxonomy of Foraminifera; many problems are remaining unsolved. Yet I believe to have proved, that only the knowledge of bionomics (statistical analysis) together with the knowledge of minor structures and especially of inner structures of the tests of Foraminifera may clear up the entanglement, in which nowadays systematics are found. I could prove, that the only reliable method of studying Foraminifera, both recent and fossil species, is the study of the test in a clarifier, for which purpose I have mainly used rhicinus-oil; and that to give a clear idea of the inner structure one has to section the tests, often with a method, which I called the glass-plate method, which also has been used with very good results in embryology. A very large and important group of Foraminifera is provided with the so-called toothplate, the function of which may be different in the different groups, but the homology of which in its different aspects was clearly shown. The toothplate and its deriving structures are very important characters in respect to taxonomy, as are the pores, both of which have been nearly totally neglected, which neglect has given rise to a confusion which nowadays is rather exasperating.

I hope, that many students in Foraminiferology will take at heart the suggestions, given in both papers mentioned; for, if new species of Foraminifera will be studied with a clarifier and with the aid of sections, together with an analysis of bionomics, it will lead to a much more reliable system, and it will be much easier to determine the species. Especially in respect to the fact, that nowadays the Foraminifera are used in a very intensive way to analyse stratigrafics, the establishment of a reliable taxonomy of Foraminifera is important and indispensable.

SCHEME OF RELATIONS BETWEEN SOME MORE
IMPORTANT FAMILIES OF THE ORDO OF
THE FORAMINIFERA, NAMED THE
DENTATA



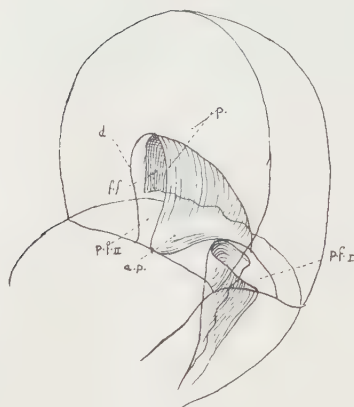


Fig. 1. Toothplates of two adjacent chambers of *Cassidulina*.
 d. distal border
 p. proximal border
 p.f.II protoforamen of former chamber
 f.f. free folded part
 a.p. attached part

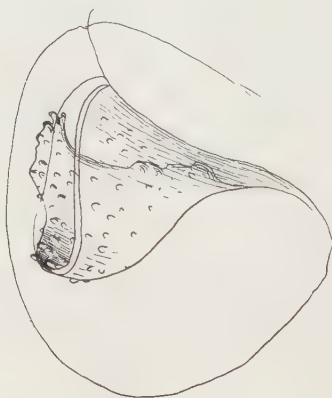


Fig. 2. Toothplate of a species of *Bitubulogenerina*, belonging to the Buliminidae, showing highly ornamented plate with protruding tongue.

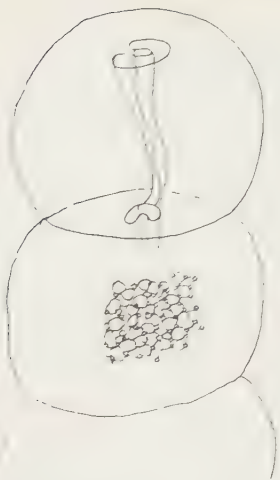


Fig. 3.
End-chambers of *Listerella spec.*, with toothplate running through chamber (consisting of chitinous matter) and pores between the sandgrains on the wall.



Fig. 4.
Apertural face of *Buliminella*, showing a poreless, radially grooved part, connected with and formed by the attached part of the toothplate, seen within last-formed chamber.

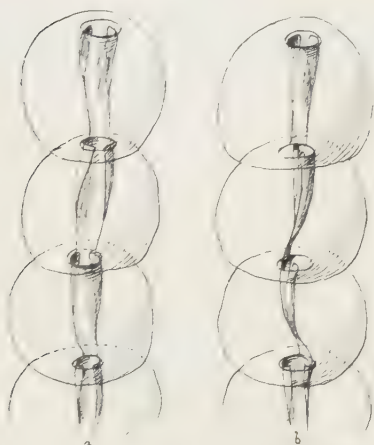


Fig. 5. a. Uniserial species (*Rectobolivina*) deriving from biserially arranged forefathers (angle between successive toothplates 180°); b. Uniserial species (*Siphogenerina*) deriving from triserially arranged forefathers (angle between successive toothplates 120°).



Fig. 6.
 A_1 -specimen of *Bulimina trigonalis* Ten Dam, showing fine protopores in the initial part and coarser pores in later chambers. $\times 240$.



Fig. 7. *a. Gavelinella intermedia* from the Albien, ventral side, with fine pores. *b. Gavelinella baltica* from the Cenomanien, ventral side, with larger pores. *c. Gavelinella daini* from the Hervien, ventral side, with very large pores, erroneously described as sandgrains.



Fig. 8. Pores of Globigerinidae. *a. Globigerina infracretacea* Glaessner from the Albien; pore-index: $17\frac{1}{2}$. *b. Globigerina pachyderma* from the North-Atlantic, with primitive characters; pore-index: $12\frac{1}{2}$. *c. Globigerina sacculifera* from the Carribean Sea, a highly specialised form; pore-index: $3\frac{1}{2}$ all $\times 480$. The pore-index is found by adding the size in mm ($\times 480$) of the pores to the number of pores ($\times 480$) found in a square cm.

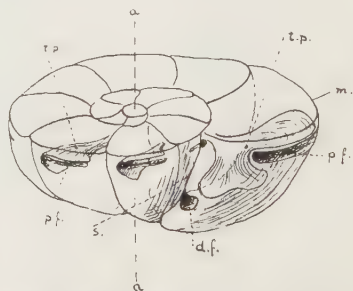


Fig. 9. Schematic figure of organisation of primitive (cretaceous) *Epistominina* s. ventral suture. p.f. protoforamen. d.f. deutoforamen. t.p. toothplate, seen through the shellwall. m. margin. a-a. axis of test. The figure shows, that the toothplate is attached to the proximal (in respect to place of proloculus) border of the protoforamen; the latter is more proximally placed than the deutoforamen, and the latter more axially, always at the suture. The protoforamen is situated parallelly to the margin and more marginally than the deutoforamen. The deutoforamen is formed at the moment of adding a new chamber.



Fig. 10. *a. Parrella*, showing the marginal and proximal place of the protoforamen, in this primitive genus of the Cibicidae connected with a small toothplate. *b. Cibicides*, the toothplate obliterated, but the situation of the foramen, proximal and marginal, still indicating its protoforaminiferal nature.

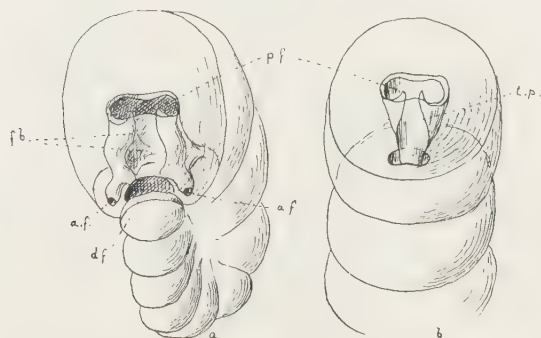


Fig. 11. *a. Cushmanella browni* with its toothplate drawn in the last-formed chamber. p.f. protoforamen. a.f. accessorial foramen, connected with canals, formed by toothplate. d.f. deuteroforamen. f.b. free folded borders of toothplate. *b.* For comparison a uniserial Protoforaminate species (*Rectobolivina*).

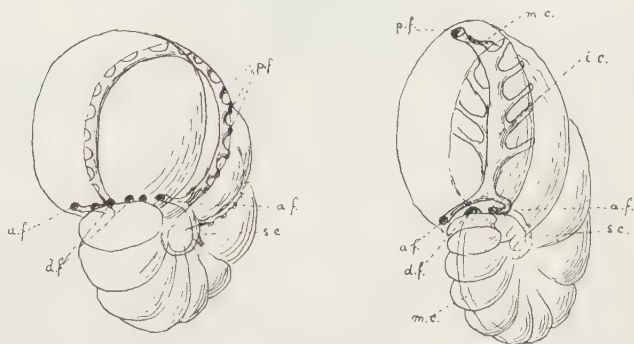


Fig. 12. *a. Elphidium*, with canal-system deriving from toothplate of *Cushmanella* (or allied genus). *b. Camerina*, with canal-system deriving from toothplate of *Cushmanella* (or allied genus). a.f. accessorial foramen, the end of spiral canal. p.f. protoforamen, in *Elphidium* forming the sutural openings, in *Camerina* the end of the marginal Chord system (m.c.). i.c. interseptal canals. s.c. spiral canal. d.f. deuteroforamen.



Fig. 13. *a.* retrai processes of next chamber between the openings of septal canal system at the suture of *Elphidium*. *b.* solid pillars without pores at the sunken suture, suggesting septal canal system, of *Elphidionion* (always with much larger pores too).

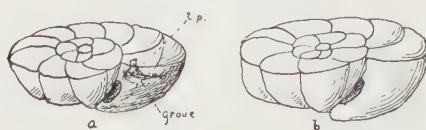


Fig. 14. *a.* *Alabamina*, with well-developed deutoforamen, whereas the protoforamen has been reduced into a closed groove in the apertural face, toward which a solid pillar-like toothplate is still running (t.p.). *b.* *Gyroidina*, showing only the deutoforamen, while protoforamen and toothplate are totally lacking.



Fig. 15. *Laticarinina*, with its toothplate developed into a broad carina and only the sutural ventral foramen left open (deutoforamen, d.f.) t.p.: toothplate.

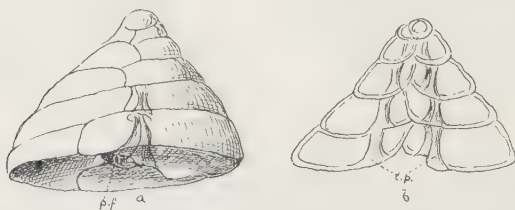


Fig. 16. *Conorbis* from the Lias. *a.* total specimen, showing at concave base the protoforamen (p.f.) and the toothplate seen through the wall; *b.* longitudinal section with toothplates (t.p.).

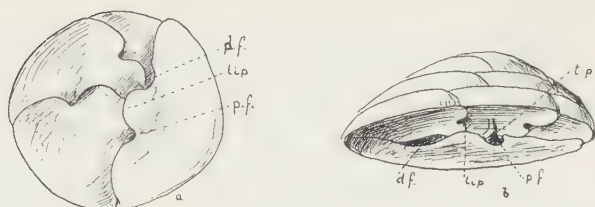


Fig. 17. *a.* ventral face of *Neoconorbina*, showing at suture of last-formed chamber the protoforamen (p.f.), the lip, and the deutoforamen (d.f.). *b.* side-view, showing through the wall the reduced toothplate, connected with the lip.

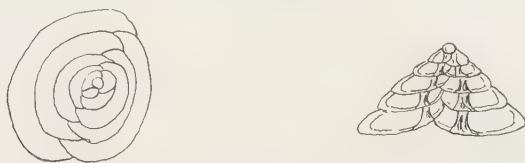


Fig. 18. *a.* Initial part of *Discobolivina* (*Patellina*), seen from above. *b.* Same in section, showing toothplates.



Fig. 19. *Reinholdella dreheri* from the Dogger, with not yet closed protoforamen (p.f.) and deutoforamen (d.f.) in last-formed chamber, and porous plates (p.p.) over the protoforamina of older chambers; ventral side.

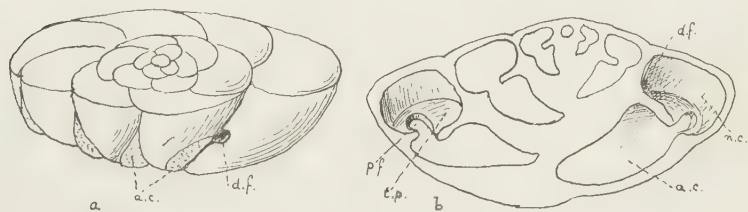


Fig. 20. *Asterigerina*. *a.* total test, with accessorial chambers (a.c.) and combination of proto- and deutoforamen (d.f.). *b.* section, showing normal chambers (n.c.) and accessorial chambers (a.c.), toothplates forming septa between normal and accessorial chambers (t.p.) with protoforamen in it (p.f.), d.f.: deutoforamen.

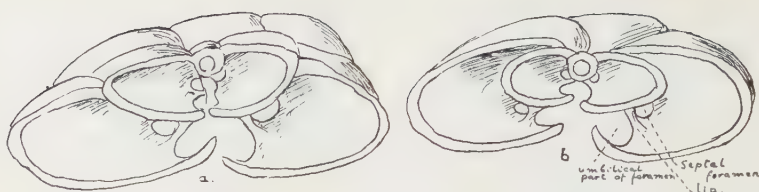


Fig. 21. *Discopulvinulina*. *a.* section through a species with all chambers visible at dorsal side. *b.* section through species with chambers dorsally overlapping; a comparison with fig. 10 *b.* at once reveals the different situation of the foramen.

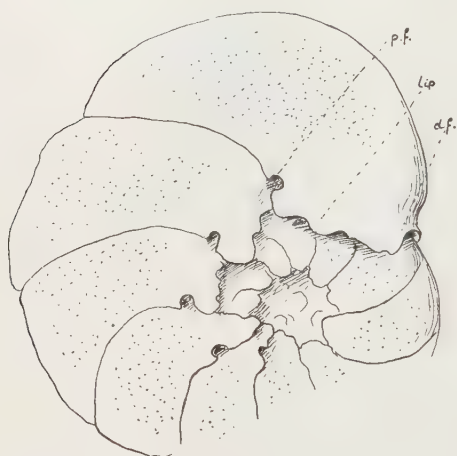


Fig. 22. Ventral side of *Discopulvinulina*, a primitive species with fine pores, showing at suture the protoforamen (p.f.), much reduced, the lip and the deutoforamen (d.f.), which, as the species is an overlapping one, secondarily is displaced toward the margin.



Fig. 23. *Cancris*, a typical genus of the *Marginolamellidae*, in which the connection between the poreless prominent lip and the poreless margin (m.) is clearly shown.

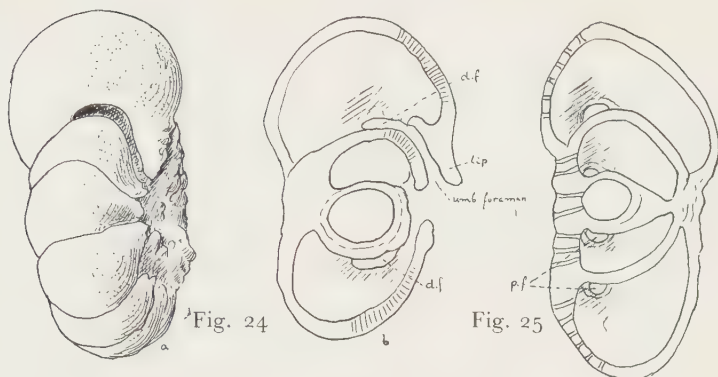


Fig. 24. *Gyroidinoides nitida* from the Upper-Gault, showing protopores only at ventral side and typical open lips connected with deutoforamen, the lips without pores. *a.* apertural face, *b.* section. d.f. deutoforamen. umb. foramen: umbilical opening of deutoforamen under the lip.

Fig. 25. *Cibicides succedens* Brotzen from the Paleocene; transverse section, megalospheric, without typical thick budd of chalk at dorsal side. A comparison with the section of fig. 27 at once makes clear, that the protoforamen (p.f.) of *Cibicides* has nothing to do with the deutoforamen (d.f.) of *Gyroidinoides* or *Gavelinetta*. In both figures the ventral side is the right one. Moreover the pores (deuteropores) here are situated mainly at the dorsal side.

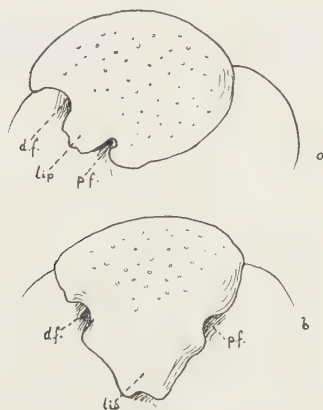


Fig. 26. *a.* Chamber, ventral side, of *Discopulvinulina*, or young *Cymbaloporetta*. *b.* Chamber, ventral side, of adult *Cymbaloporetta*. d.f.: deutoforamen. p.f.: protoforamen. The deutoforamen always is situated at the distal end of the chamber.

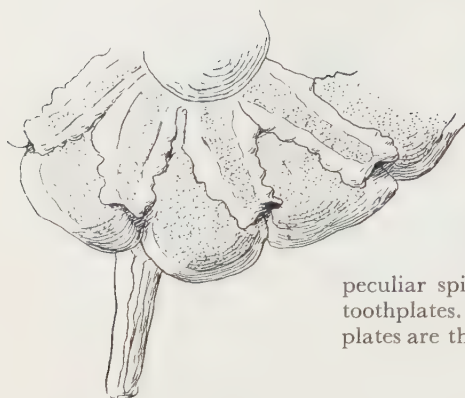


Fig. 27. Ventral side of part of *Asterorotalia pulchella*; the sutures are partly covered by porous plates, which cover the hollow between these plates and the sunken sutures and open near to the margin. The peculiar spines showed to be lengthened toothplates. $\times 240$. Underneath these porous plates are the openings of the protoforanina.

Fig. 28. *Buliminella milleti* Cushman, from Lasquiti-Island. Two copulating A_2 -specimens (proloculus-diameter 43μ). The pores of walls only indicated at one of the chambers; plasmodiospores or rather gametes are found in multitude within the specimens, in which all septa are absorbed. In any case it is obvious, that the offsprings of those specimens will begin with a much smaller proloculus, probably microspheric.

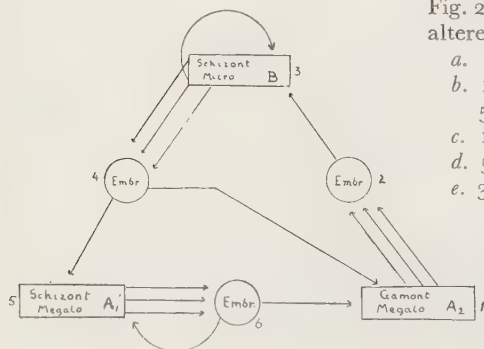
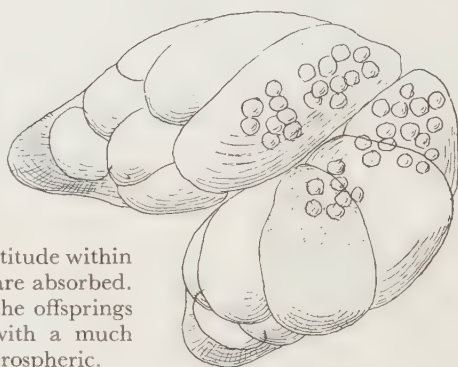


Fig. 29. Reproduction-scheme, slightly altered after LE CALVEZ.

- a. 1-2-3-4-5-6-1: holotrimorphic.
- b. 1-2-3-4-5-6-1 + 1-2-3-4 + 5-6-5: paratrimorphic.
- c. 1-2-3-4-1: dimorphic.
- d. 5-6-5: apogamic.
- e. 3-3-3: apogamic (endomixic).

a and b are found in primitive species mostly; c in more advanced species, whereas d and e occur in highly specialised forms, e especially in pelagic species.

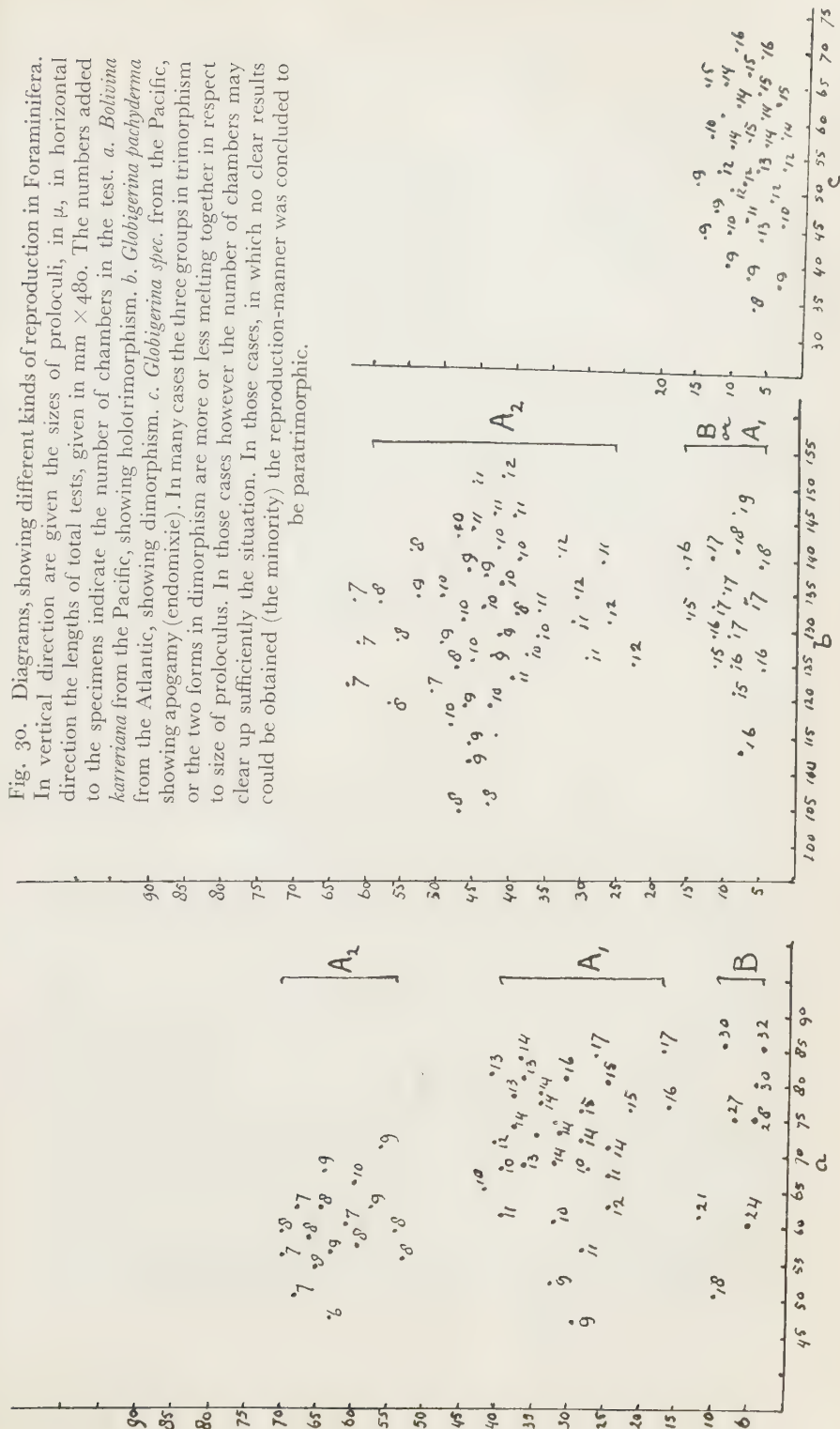


Fig. 30. Diagrams, showing different kinds of reproduction in Foraminifera. In vertical direction are given the sizes of proloculi, in μ , in horizontal direction the lengths of total tests, given in mm $\times 480$. The numbers added to the specimens indicate the number of chambers in the test. *a. Bolivina karriana* from the Pacific, showing holotrimorphism. *b. Globigermia pachyderma* from the Atlantic, showing dimorphism. *c. Globigermia spec.* from the Pacific, showing apogamy (endomixie). In many cases the three groups in trimorphism or the two forms in dimorphism are more or less melting together in respect to size of proloculus. In those cases however the number of chambers may clear up sufficiently the situation. In those cases, in which no clear results could be obtained (the minority) the reproduction-manner was concluded to be paratrimorphic.

LE PROBLÈME DU TONUS ET QUELQUES- UNES DE SES DIFFICULTÉS. LA DÉCONTRACTION SUSPENDUE ¹⁾

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Par définition le tonus est la tension basale présente dans les muscles pour autant qu'elle est indépendante de la volonté.

Comme toute autre tension, le tonus peut s'exprimer par le nombre de grammes que le muscle est en mesure de supporter. La longueur du muscle dans cet état statique fournit une autre donnée. Il est absolument inadmissible que l'on confonde ces facteurs dans un raisonnement. Dans des circonstances physiologiques l'adaptation des intestins à l'état de plein est telle, que la tension reste la même à divers degrés de raccourcissement. — Nous connaissons la même dualité dans les muscles squelettiques: il est possible de porter le même poids avec un bras court et un bras long.

L'usage d'autres normes sans distinctions dans des considérations théoriques — constituant une autre source de confusion — est produit par des différences dans la manière dont l'équilibre se produit entre la charge et la tension du muscle. Cet équilibre s'établit rapidement dans le muscle squelettique; il faut passablement de temps au muscle intestinal pour y atteindre. Dans ce dernier cas, on s'est avisé de décrire le tonus par l'inclinaison du myomécánogramme qui indique le cours du processus ou par celle de la tangente de cette courbe. On définit alors le tonus par l'expression: résistance relative à l'allongement. Plus l'angle formé par l'oblique est grand, plus le tonus est faible. Il ne faut pas non plus confondre ce facteur avec celui de la longueur: quand deux courbes d'allongement s'intersectent, la résistance des deux côtés du point d'intersection présente les mêmes proportions tandis que, à en juger par la longueur, il s'est produit un changement.

Quant au comportement mécanique du muscle, la dureté du

¹⁾ Exposé pour la Société Néerlandaise de Zoologie à sa séance du 8 mars 1947 à Leyde, augmenté de quelques considérations sur des communications plus récentes (10, 22 et 25).

muscle peut aussi servir de point de comparaison (NOYONS et von UEXKUELL, 27).

En ce qui concerne les réactions physiologiques qui constituent le tonus, la définition ne nous apprend rien de plus que ce qu'elles peuvent se produire hors de notre conscience. Il ne peut y avoir de confusion aussi longtemps qu'on admet un mécanisme uniforme pour les différents types de muscles (muscles squelettiques, muscles du tube digestif et muscles lisses rubaneux). On s'est toujours efforcé d'arriver à une conception moniste de ce genre, d'après laquelle le tonus serait un tétanos parfait (tetanotonus), alternant d'une fibre à l'autre. Le muscle ne parvient pas à un tétanos maximal: pour chaque fibre, des périodes d'activité alternent avec des périodes de repos, ce qui explique pourquoi il ne se produit pas d'épuisement. Comme le tétanos parfait est réduit à une sommation et une fusion complète de secousses successives, ce processus exige un surplus d'énergie en raison de son intensité, comme l'indique le métabolisme accru.

On a protesté à plusieurs reprises contre une pareille unification de tous les types de muscles – BETHE (5), BIEDERMANN (7), JORDAN (17) –. C'est pourquoi on a distingué des mécanismes essentiellement différents pour les muscles lisses et pour les muscles striés, en vertu des arguments suivants:

1. le tracé myographique du tétanos provoqué aux muscles intestinaux par excitation tétanique ne présente pas un tableau horizontal; les muscles se fatiguent trop vite.

2. leurs prestations toniques ne sont pas accompagnées par une intensité équivalente des échanges respiratoires.

C'est pourquoi BIEDERMANN a formulé l'opinion suivante: "Gibt man zu, dass die Erschlaffung nicht einfach nur die Folge des Aufhörens der Erregung ist, sondern auf dem Neueintreten eines zweiten activen Vorganges beruht, welcher dem ersteren (dem Contractionsprocess) in jedem Sinne entgegengesetzt ist (ce qui veut dire: "der bisher üblichen Auffassung" – 1904 –), so ist klar, dass der verkürzte Zustand so zu sagen fixiert werden kann, wenn jener Erschlaffungsprocess gar nicht oder nur unvollkommen einsetzt". (7, p. 517). On admit alors que le raccourcissement et le rallongement subséquent des muscles non striés sont deux phénomènes actifs indépendants, donc à effet contraire provoqués séparément par un influx spécifique: "la décontraction

commandée annule la contraction" (LEFÈVRE 22 p. 297). A côté du tetanotonus on constata l'existence d'un tonus dit tonus à cliquet. (terme employé par von UEXKUELL - 34 - mais appliqué par lui à un autre mécanisme).

Les monistes pensent pouvoir attribuer les phénomènes contraires du tonus résumés ci-dessus à la lenteur de la secousse des muscles lisses (sa durée peut comporter six cents fois celle de la secousse des muscles striés rapides) ce qui expliquerait pourquoi moins de stimulations tétaniques sont nécessaires par unité de temps que pour les muscles squelettiques. Il ne serait donc pas question d'une intensification mesurable des échanges respiratoires (BAYLISS, 4) non plus que d'une impuissance causée par l'épuisement.

JORDAN s'était d'abord (16) rallié à ceux qui considéraient le tonus à cliquet comme l'agent de la tension involontaire des muscles lisses. Cependant, comme JORDAN en vint à croire qu'il n'était pas possible de provoquer une chute du tonus par le moyen d'une excitation, il a attribué plus tard cette fonction à la structure submicroscopique des éléments musculaires, écartant ainsi la contraction (17, 18) et toute autre réaction physiologique.

Relativement à ce "tonus de structure", JORDAN distinguait des centres (micelles) à réaction élastique dans une masse visqueuse et amorphe; cette masse intermicellaire constituerait une résistance qui amortirait l'activité élastique des centres (viscosité ¹) parallèle) et qui s'opposerait par refoulement au glissement des micelles (viscosité ¹) combinée par série). Finalement, l'allongement ayant atteint un certain degré, les éléments élastiques agiraient les uns les autres, ce qui ferait à son tour croître la résistance à l'allongement (la "consolidation", cf. 30 p. 398).

L'existence de cette conformation structurale a été finalement établie en vertu des phénomènes d'allongement et de recouvrement décrits ci-dessous:

1. Le recouvrement qui suit le déchargement du muscle diminue sous l'influence de la chaleur: la masse intermicellaire devient plus liquide ce qui la rend moins apte à réintégrer son logement primitif.

2. Quand l'allongement est rapide, les micelles de la masse

¹) de là le tonus dit "viscosoid" ou "visqueux" (JORDAN, 19).

intermicellaire subissent une "accumulation" ou "effet de chasse-neige" (cf. 30, p. 398) qui se résout durant une pause, lorsque l'allongement est intermittent.

3. Quand on fixe les deux extrémités du muscle l'on constate que la tension provoquée disparaît presque entièrement: la tension élastique des micelles se résorbe à l'intérieur de la masse intermicellaire ("relaxation isométrique").

Dans ses considérations théoriques, JORDAN n'a pas marqué suffisamment les différences entre les facteurs cités plus haut (la résistance et la longueur). La confusion qui en résulte est encore augmentée par le fait que JORDAN dans ses publications ne tenait pas toujours compte de l'acception précédemment faite par lui-même de certaines modifications apportées à sa théorie sur le tonus "viscosoid", de sorte qu'il est difficile de constater quelles théories – de son propre avis – ont fait place à de nouvelles hypothèses. Le problème du tonus a pris par là un aspect très compliqué.

Nous ne désirons pas étudier ici plus profondément cette théorie sur le tonus; pendant l'occupation allemande nous avons réussi à démontrer que, dans le pied de l'escargot, formé par des muscles lisses, le tonus est constitué par la relation entre des éléments musculaires contractés et des éléments décontractés, exactement comme pour les muscles squelettiques. Ce tonus est déterminé à son tour par la relation entre les impulsions antagonistes particulières, mais alors dans les voies nerveuses efférentes. C'est pourquoi nous nous sommes ralliés à l'ancienne conception dualiste (30); nous distinguons donc deux mécanismes du tonus qui utilisent différemment les éléments de contraction: le tétanos à contraction répétée intermittente et le tonus à cliquet à décontraction suspendue ¹⁾. Tandis que dans le tétanos les influx antagonistes agissent sur le système nerveux central et que de leur relation dépendent des impulsions qui atteignent le muscle et provoquent les contractions, le tonus à cliquet naît de la suspension de la décontraction, causée par l'absence de l'excitation qui doit porter sur le muscle. La décontraction ne se produit ici donc pas spontanément comme au muscle strié. Dans ce dernier cas il faut même qu'elle soit constamment annihilée par des impul-

¹⁾ LEFÈVRE, 23 p. 297.

sions tétaniques plus ou moins fréquentes; d'où l'"onéreuse" activité phasique.

VAN KOERSVELD collaborant avec KRIJGSMAN, défend encore (1949) la thèse du tetanotonus, mécanisme du pied de l'escargot, bien que soit "sous une forme modifiée" (22, thèse I), en se basant sur les constatations suivantes:

KRIJGSMAN a noté une diminution des influx actifs des nerfs pédaux quand les ganglions sub-oesophagiens sont soumis à une excitation chimique (les expériences en 1942 n'ont pas encore été publiées en extenso; cf. POSTMA 29, p. 188). (Utilisant une telle excitation dans d'autres expériences, nous-mêmes avons obtenu une tonolyse 28). Le ganglion cérébral subissant à son tour une excitation chimique, VAN KOERSVELD a découvert qu'il existe un parallélisme entre le niveau du tonus et le nombre de contractions spontanées observées p. ex. dans l'espace de dix minutes. De plus une certaine fréquence des contractions accompagnait un niveau déterminé du tonus, que le changement du tonus se produise spontanément ou qu'il soit provoqué par une stimulation arbitraire, C'est pourquoi ces expérimentateurs ont défendu la théorie monistique. KRIJGSMAN attribue le fait que les ganglions pédaux règlent le tonus, au nombre des influx excitatrices (partant du centre dorsal — cf. 20) que le centre ventral régulateur laisse passer. C'est-à-dire qu'il l'attribue à une inhibition centrale telle que celle du système spinal des vertébrés et non pas à une tonolyse périphérique, comme nous l'avons dit ¹⁾. D'ailleurs nous ne pouvons pas prétendre que le tonus à cliquet règne seul dans le pied de l'escargot; c'est pourquoi nous avons été prudents dans nos déclarations (POSTMA 31 p. 490/91) citées erronément par VAN NIEUWENHOVEN (25 p. 106), un autre collaborateur de KRIJGSMAN.

Les recherches de VAN NIEUWENHOVEN avaient pour but de découvrir si le muscle strié et le muscle lisse fonctionnent réellement de manière aussi dissemblable à l'égard du tonus que les dualistes le soutiennent. Il se servit à cet effet du rétracteur byssus de *Mytilus edulis*, un muscle lisse étudié par l'école de WINTON (35) et qui réagit en unité. Suivants les traces de JORDAN, VAN NIEUWENHOVEN a étudié en premier lieu les phénomènes d'allongement et de recouvrement. Celui-ci étant intensifié par la chaleur, l'accumulation et la relaxation isométrique faisant

¹⁾ Nous remercions M. VAN KOERSVELD (les expériences faites en 1943-1946, mais pas encore publiées) de nous avoir autorisé à publier ce résumé.

défaut, VAN NIEUWENHOVEN est forcé de conclure quant à l'objet de ses recherches à une structure entièrement opposée à celle que JORDAN attribue à ses objets: micelles plastiques entourées d'une masse intermicellaire à réaction élastique. VAN NIEUWENHOVEN attribue cette déformation plastique à la transformation longitudinale α à β décrite par ASTBURY (1) des molécules de myosine. Selon VAN NIEUWENHOVEN lui même, la charge nécessaire pour provoquer ces phénomènes de déformation est si formidable et l'amplitude obtenue est si minime que pareille occurrence ne se produit que bien rarement dans la vie de l'animal et ne joue aucun rôle dans la physiologie: "we think that this last (α - β transformation) seldom happens in life" (p. 84). On dénie donc ici l'importance fonctionnelle du "tonus structural".

Ce point de vue s'harmonise avec l'opinion de JORDAN. Précisément le fait que ce muscle lisse rubaneux, de même que le muscle squelettique strié manifeste une élasticité presque parfaite dans son recouvrement total, a constitué aux yeux de JORDAN, jusqu'à la deuxième guerre mondiale, une raison pour ne jamais faire entrer dans ses commentaires les résultats obtenus par WINTON et FLETCHER. D'après lui, une manifestation mécanique de ce genre démontrait que, dans les deux cas, un certain degré de raccourcissement devait être maintenu par le moyen de tetanotonus — ceci en opposition avec les muscles lisses creux, en forme de poche, étudiés par JORDAN.

En second lieu VAN NIEUWENHOVEN a confirmé les observations de FLETCHER, savoir qu'il est possible — selon le genre et le lieu de stimulation — de provoquer une contraction accompagnée d'une relaxation spontanée du rétracteur byssus ou même une "contraction à cliquet", c'est-à-dire: un état prolongé de raccourcissement qui ne cesse que lorsqu'il est interrompu par une excitation spéciale provoquant la décontraction.

Une excitation faradique du muscle relâché a pour résultat la contraction phasique citée plus haut. Une excitation faradique intensifiée du ganglion pédal, ou une galvanisation du muscle, conduit à la "postural contraction" (FLETCHER). Une faible excitation faradique du ganglion ou une excitation galvanique du muscle contracté, rétablit l'état détendu. VAN NIEUWENHOVEN déclare effectivement que ce mécanisme est responsable des changements de longueur physiologiques fonctionnels qu'a subis le muscle, ainsi que de leur persistance se rapprochant ainsi de l'hypothèse que nous avons adoptée plut tôt quant au pied de

l'escargot. Il les attribue à la "super-contraction" signalée par ASTBURY (2), avec sa transposition de la structure longitudinale α à la structure transversale β (RUDALL 32). Suivant VAN NIEUWENHOVEN, cette contraction – que l'on peut provoquer séparément de la décontraction – a plus d'importance que le tétanos pour un fonctionnement "économique" du tonus. L'erreur commise en ignorant les recherches de BIEDERMANN e.a., se manifeste dans la conclusion de sa dissertation: "We must therefore come to the conclusion that JORDAN's fundamental idea of explaining the tonus of smooth muscles by their sub-microscopic structure need not be given up at all, ..." (p. 109). Voilà qui porte à confusion dans une forte mesure: VAN NIEUWENHOVEN n'est évidemment pas au courant de l'origine de la controverse JORDAN-BIEDERMANN: ces deux savants n'étaient pas d'accord principiellement sur le fonctionnement de la structure. Le tonus viscosoïde de JORDAN est incompatible avec la contraction à cliquet. Il est également faux d'identifier cette dernière (25 p. 98) avec ce que JORDAN appelait: la contraction tonique; JORDAN entendait par là un raccourcissement lent (en comparaison de celui qui s'opère par secousse), causé par un reflux de la masse intermicellaire, donc se distinguant nettement du mécanisme de la contraction phasique rapide (Cf. L. M. VAN NIEUWENHOVEN, 25, p. 102 fig. 15; pas de différences entre les tracés ascendants),

Selon la théorie du tonus à cliquet la relaxation (suspendue) est un phénomène actif. "Par conséquent il vaudrait mieux parler de décontraction que de relaxation" ¹⁾. Nous ne connaissons pas encore le mode d'action de l'influx spécifique, ni le mécanisme de la décontraction. Suivant les nouvelles théories sur la secousse, l'élément musculaire détendu présente un niveau d'énergie plus élevé que l'élément contracté. On pourrait le comparer à un ressort tendu par des champs électriques; la contraction constitue la réduction du degré d'extension (BETHE, 6) et peut donc se produire passivement ²⁾ en conséquence d'un affaiblissement des champs (BUCHTHAL, 9). La restauration de leur intensité se

¹⁾ D'après le manuscrit inédit d'un discours (1944) à la "Commission pour l'étude de la Viscosité" instituée par l'Académie des Sciences d'Amsterdam. Cf. aussi: E. GLEY (14, p. 618, mais sans cet exposé du motif) et POSTMA 30 p. 398 et 31 p. 472).

²⁾ Cf. l'opposition de A. V. HILL, 1949, Proc. Roy. Soc. London B **CXXXVI**, p. 420 (Is relaxation an active process?); 1950, Ibid. **CXXXVII**, p. 40 (A discussion on muscular contraction and relaxation).

manifeste par la descente du myomécanogramme et doit être couverte par quelque métabolisme. Nous en connaissons trois cycles: celui du glycogène, celui de l'acide créatine-phosphorique (ou phosphagène) et celui de l'adénosinetriphosphate (ou A.T.P.).

On concède généralement que le métabolisme du glycogène, conduisant à la production d'acide lactique, soit en relation directe avec le raccourcissement (24) et uniquement avec celui-ci, tandis que la relaxation s'opérerait passivement. Les recherches de SZENT GYÖRGYI (33) nous offrent deux points de contact: la mise à disposition de ions de magnésic (via ceux de calcium) et le déblocus de l'activité phosphatasique du complex d'actomyosinate par rapport au métabolisme d'A.T.P., la réactivation de la phosphatase qui par une réaction hydratante thermo-osmotique conduirait à la "relaxation". SZENT GYÖRGYI ne se sert pas du terme "decontraction" (Cf. 25 p. 107); le Hongrois ne tient pas compte de l'activité ni de la passivité du raccourcissement ou du rallongement.

VAN NIEUWENHOVEN attribue l'absence de relaxation succédant à la contraction à cliquet, aux phénomènes décrits par EGGLETON (11) sous le nom de "exhaustion phenomena" et qui lui font supposer une interruption des réactions phosphorantes de la glycogenolyse (cf. PARNAS, 26 et VAN HERK, 15). Une déficience d'A.T.P., causerait la suspension du processus de restauration et la contracture du muscle. VAN NIEUWENHOVEN oublie qu'un muscle empoisonné par l'acide mono-iodascétique – état dans lequel le cycle du glycogène est bloqué – peut encore exécuter environ 70 secousses musculaires avant qu'il soit question d'épuisement et de contracture. D'après les données fournies par SZENT GYÖRGYI, une insuffisance de restauration par l'A.T.P. ne jouerait un rôle décisif qu'après 35 à 50 secousses; il n'est donc pas admissible que l'A.T.P. soit épuisé après un seul et unique raccourcissement, causant ainsi la contraction à cliquet.

L'interprétation de DUBUISSON (10) se rapproche davantage de la théorie de BIEDERMANN; DUBUISSON attribue la contraction à l'hydrolyse de l'A.T.P., la décontraction à celle de la phosphocréatine. Il admet donc l'activité du muscle dans les deux phénomènes.

Il n'est pas encore possible de choisir entre ces différentes hypothèses. On peut toutefois faire abstraction de ces points de controverse à l'égard de la théorie, admise aussi par JORDAN (21) en tant que le caractère réactif et réflectoir, qu'il faut reconnaître à côté d'un tetanotonus un tonus' à cliquet.

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ON THE INNERVATION OF THE MUSCLES IN THE LEG OF THE LOBSTER, *HOMARUS VULGARIS* MILNE EDWARDS

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INTRODUCTION

Previous work made it possible to determine the innervation scheme of the leg muscles for different divisions of the decapod Crustaceans (WIERSMA, 1941, 1948). The number of single nerve fibers which innervates any of these muscles is always small. Thus it was found that the 7 muscles of the three peripheral joints of the leg of *Panulirus*, the rocklobster, are innervated by only 15 axons, of which 12 are motor, whereas 3 are inhibitors. These few fibers must regulate all the movements for which the lower part of the leg can be used. In other subdivisions of the Decapods the number of fibers has been proved to be the same, though the distribution of the inhibitory fibers is different. It seems likely that this difference in distribution corresponds with the subdivisions of the Decapods, and it may therefore be of importance also from a systematic point of view. For the Astacura the innervation scheme was almost entirely based on the findings in freshwater forms, *Astacus* and *Cambarus*. Though *Homarus* has been little investigated from this aspect, it may be concluded from the works of HOFFMANN (1914), KNOWLTON and CAMPBELL (1930) and WIERSMA (1933) that at least in some important points the innervation of both *Homarus vulgaris* and *Homarus americanus* is like that in the crayfishes. However, in the latter, though it had proved possible to determine the innervation of the four most peripheral muscles without undue difficulty, the innervation of the muscles in the meropodite remained incompletely known, since the length of nerve available for isolation of single nerve fibers was insufficient in the ischiopodite to allow definite separation of all fibers concerned. Hence, the present investigation was undertaken in order, on the one hand, to determine whether the innervation of *Homarus* was in all respects the same as for the known part of the

muscles in *Cambarus*, and on the other hand to extend the scheme, if possible, to those muscles which were not satisfactorily investigated in the crayfishes.

METHOD

The method used has been extensively described in a previous paper (WIERSMA, 1941) and will not be repeated here. As was pointed out, it is necessary, in order to obtain a complete picture of the innervation of these muscles, to isolate all motor and inhibitory axons and to stimulate them separately. The relatively small number of axons innervating each muscle, a number, which has so far been found to vary only between two and five, makes this procedure feasible. For this purpose micromanipulated electrodes are used, of which one pair suffices for the determination of the number and function of motor axons, but of which two pairs must be used for the location of inhibitory fibers.

In the present investigation all nerve fiber isolations have been carried out in the ischiopodite. This procedure has the advantage that in every preparation also the innervation of the muscles in the meropodite could be investigated. In many cases subsequent staining with methylene blue was performed. It was found, that by carefully freeing the muscle from the shell such staining could give good results on isolated muscles. This method has the advantage above the more conventional one in which one muscle of each joint is discarded and the other stained attached to the shell, that both muscles from each joint can be stained. The results were as good and sometimes better than with the other method. In both instances great care must be taken not to damage the innervated side of the muscle on which the arborization of the nerve fibers takes place, as otherwise at best partial staining of the axons is obtained.

RESULTS

The motor innervation

The number and function of the nerve fibers which cause, on stimulation, contraction of the muscles was found to be the same as in all other Decapods, thus far investigated. Two main nerve bundles are present, both in the ischiopodite and in the meropodite. Each of these contains a number of motor fibers, which

are usually again assembled so that only a very small part of each main bundle causes any motor effect on stimulation. In the thicker main bundle are motor fibers for the closer (adductor of the dactylopodite), the bender (flexor of the propodite), the extensor (of the carpopodite) and the accessory flexor (of the carpopodite). In the case of the latter muscle the motor effect is sometimes obtained by stimulation of a different part of the main bundle than the one which causes the other movements. In other cases, however, none such bundle can be found, and it is then necessary to separate the extensor motor axons from the remaining motor fibers before the flexion of the carpopodite can be observed. If the thinner of the two main bundles is stimulated, contractions of the opener (abductor of the dactylopodite) and of the main flexor (of the carpopodite) are regularly noticed, whereas contraction of the stretcher (extensor of the propodite) occurs often only on much stronger stimulation.

By further splitting these bundles of fibers it can be determined how many motor fibers each separate muscle receives. In the main bundle are thus found two motor fibers causing contractions of the closer. These two contractions differ clearly in speed when the nerve fibers are faradically stimulated. In the present investigation it was not possible to study such differences extensively, but from previous experience it is known that such differences prove the presence of fast and slow contractions. Older experiments (WIERSMA, 1933) tended to show, that in *Homarus* the fast closer contraction seems to be speedier in the sharp cutter than in the blunt crusher claw. In the present investigation most experiments were carried out on the 2nd to 4th thoracic legs, claws being only occasionally used. In one instance a cutter claw was investigated with two induction shocks applied to the fast fiber. Single shocks had very little effect, in conformation of the findings with short lasting direct current shocks (WIERSMA, 1933), and in contradistinction to *Cambarus*, in which a single nerve impulse in the fast fiber is sufficient to cause a complete quick closing of the claw. However, two shocks in quick succession do cause in the cutter claw a very powerful contraction; the claw closes with a snap. With slightly longer intervals between shocks the contraction diminishes quickly in height, and disappears when the shocks are somewhat further apart. The exact time intervals could not be determined with the apparatus at hand, but they are of the order of milliseconds. This picture is

similar to that obtained in certain other fast systems of closers, which have been more extensively investigated, namely those of *Randallia ornata* and *Blepharipoda occidentalis* (WIERSMA and VAN HARREVELD 1938). It is noteworthy that the thousands of muscle fibers, which are present in these large closer muscles are all brought into contraction by the impulses of a single axon. At least in the 2nd thoracic leg single shocks did have a much more pronounced effect, whereas the administration of double shocks was relatively less effective as no very strong contractions are thus obtained. Since in the crusher claw two shocks are less effective than in the cutter claw, and single shocks are, as there, without much effect at all, it seems clear that these closers differ noticeably in facilitation of the fast contraction. When reflex contractions are caused by touching the inside of the claws, differences in speed of closing, corresponding to those on electrical stimulation, can be observed. Further experiments on these systems would seem worthwhile for obtaining more insight into the problem of facilitation of contraction.

In addition to the two motor fibers for the closer, there are present in the main thicker bundle two motor fibers for the bender and two for the extensor. The difference between fast and slow is in the bender less clear than in the extensor, which is in agreement with the findings in many other species. The nerve fiber for fast extension often causes on stimulation jerky movements of the joint, whereas those caused by the nerve fiber for fast bending are always smooth.

As mentioned above, flexion of the carpodite can also be obtained by stimulation of part of the thicker main bundle. This flexion is caused by the accessory flexor, a two bellied muscle which runs next to the main flexor. In about half the preparations this flexion is obtained by stimulation of a separate subbundle. In this case it was found on further separation that a single motor fiber was responsible for this contraction. Since the extensor contraction is stronger than that of the accessory flexor it was necessary, when the nerve fibers ran together in one bundle, to separate the two extensor axons from the flexor axon, before the contraction caused by the latter could be observed. The finding, that the accessory flexor is innervated by a single motor fiber, is again in agreement with all other cases, in which the innervation of this muscle has been determined (*Cancer*, *Panulirus*, *Birgus*).

The number of motor fibers in the thinner main bundle is less

than that in the thicker one. As in all other species a single motor fiber forms the sole motor innervation of both the opener and stretcher. No other fibers have any motor effect on these two muscles. There remain four thicker fibers, of which it has been possible to show definitely that stimulation of each of them causes contractions of the main flexor, which was not possible to prove with certainty in the crayfishes. According to the type of contraction caused by faradic stimulation it is easy to distinguish a very fast and a slow contraction. The two remaining contractions are of an intermediate speed, but are not obviously different from each other at this frequency. The size of the four fibers is not so different as in *Panulirus*.

No other motor fibers innervating these muscles have been found. The total number is the same as in all cases for which a complete picture has been obtained, and the distribution is identical.

The inhibitory innervation

The distribution of the inhibitory fibers is of particular interest, since it is known that there are here considerable differences between the different subdivisions of the decapods. Easiest to locate was the inhibitor of the stretcher, which runs, as in all other species, always alongside the opener-stretcher motor axon. As long as these two fibers have not been separated, contractions of the stretcher are therefore often completely suppressed. HOFFMANN has shown that in both *Astacus* and *Homarus* the stretcher also inhibits the closer, at least to a certain extent. In the other subdivisions this is not the case. It was found that, as expected from the experiments with *Cambarus*, this inhibiting effect on the closer is limited to slow closer contractions. In *Astacus* and *Cambarus* the inhibiting effect on even these is very small, and only noticeable, when the frequency of excitation is much lower than that of inhibition. In *Homarus* no exact determination could be made. Stimulating both fibers faradically led to some very partial inhibition. When the slow motor fiber was stimulated at a lower frequency (with a vagus stimulator) inhibition by faradic stimulation of the stretcher inhibitor became complete. One gained the impression that the slow closer was somewhat more efficiently inhibited in *Homarus* than in the crayfishes, but that here too the R_c (frequency of inhibition/frequency of excitation for just complete suppression of any contraction) is well above 1, though

lower than $4\frac{1}{2}$, the value in *Cambarus* (WIERSMA and ELLIS, 1942). No effect on any of the other muscles was found for this inhibitor, though it was carefully tested against low frequency excitation of all other slow motor fibers.

The inhibitor for the opener was known to cross to the bundle containing the closer motor fibers (HOFFMANN, KNOWLTON and CAMPBELL, WIERSMA) and could be located without undue difficulty in the thicker main motor bundle. As in the case of the stretcher inhibitor on the stretcher contractions, this fiber suppresses completely the opener contraction when both axons are faradically stimulated. No other muscles could be inhibited by this fiber. Special attention was paid to contractions caused by the slowest of the four motor fibers of the main flexor, without result.

A third inhibitory fiber ran also in this thicker main bundle, and was found to suppress completely the contractions of both the slow bender and the slow extensor. On the fast contractions of these muscles, a noticeable but not complete inhibition was found on faradic stimulation of the axons concerned. This is in agreement with the findings in most other species. Again this inhibitor was tried against the contractions of all the other muscles, since, in *Birgus*, it was found to inhibit all muscles, and in crabs, all but the main flexor. However, no effect was found on the opener, closer, stretcher and main flexor. The contractions, caused by stimulation of the accessory flexor motor axon were, on the other hand, completely suppressed. This inhibitor therefore does have an influence on two antagonistic muscles: the extensor and the accessory flexor.

The results thusfar have provided an inhibitor for six of the seven muscles, the main flexor being not inhibited by any of the three inhibitors. It was found that in the bundle containing its motor axons a thinner axon was present, which on faradic stimulation gave a complete suppression of the contractions caused by all three slower motor axons, and that even the fastest contraction was, though not suppressed, noticeably reduced. When this inhibitory fiber in turn was tried against the motor axons of the other muscles no influence at all was found.

Staining with methylene blue

In general the best results were obtained by using an intermediate concentration, which took about 1 hour for maximal

staining. The results were not very satisfactory, for, whereas the motor fibers and the inhibitors of the opener and stretcher stained readily, the other inhibitors, which are much thinner, were often not visible. The number of motor fibers checked in each instance with the one found by physiological preparation. In the main flexor two of the fibers were for some unknown reason much more difficult to stain than the other two. Nevertheless many preparations were obtained in which all 4 fibers were clearly observed to divide in the same places. A noticeable observation was that both in the extensor and in the closer an early branching of the two motor fibers was present. Each branch innervated the muscle on one side of the tendon only, though once in a while a small side branch crossing the tendon was observed. In some instances a thin fiber clearly visible in part of the preparation, was observed in addition to the two thick fibers in closer, bender and extensor. In well stained preparations two fibers were always found in the opener, and never any sign of a third one. The branching in the stretcher was often rather irregular. In some cases three fibers seemed to be present, but by closer inspection most of these showed that this was caused by a premature branching of one of the two fibers, since after the other fiber had also divided both branches contained only two fibers. Only in occasional preparations five fibers were visible in the main flexor. In the accessory flexor a rather thick fiber was regularly stained, but no trace of any other fiber could be found.

It can be concluded that the results of staining confirm in general the physiological findings, but that staining of thin fibers is too unreliable to allow much importance to it.

DISCUSSION AND SUMMARY

The innervation as described for the crayfishes has been confirmed in the lobster and it has been possible to extend the innervation scheme to more muscles. (Fig. 1).

The innervation of the main and accessory flexors are described here for the first time in an Astacuran. As expected the main flexor is innervated by 4 motor fibers and 1 inhibitory one, the accessory flexor by 1 motor and 1 inhibitory fiber. The latter was shown to be a branch of the inhibitor which supplies the extensor and the bender. It has not been possible to show that the inhibitory fiber of the main flexor is likewise a branch from

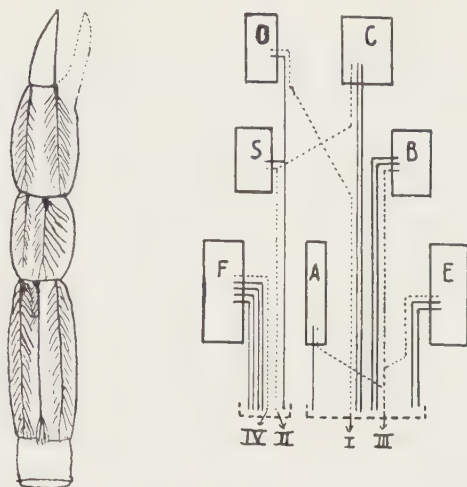


Fig. 1. On the left schematic drawing of thoracic leg, showing the location of the 7 muscles, which move the three distal joints.

To the right these muscles are represented by rectangles, having the same relative location. O – opener; C – closer; S – stretcher; B – bender; F – main flexor; A – accessory flexor; E – extensor.

Each line in the right drawing represents a single axon, all axons innervating the 7 muscles are represented. Drawn lines are motor axons, dotted lines inhibitory axons. The brackets show left the thinner and right the thicker main bundle of nerve fibers, as found in the ischiopodite.

one of the other inhibitors. Though this was the case in all other species thus far fully investigated. The question arises therefore, whether a 4th inhibitor is present in the *Astacura*, or whether one of the inhibitors branches in the proximal part of the leg, before the ischiopodite. It is not possible to decide this question. It seems unlikely that the stretcher inhibitor would branch so early, as this fiber runs in the small bundle with the main flexor axons. Again, the branch to the accessory flexor of the “third” inhibitor may be taken as an indication that the latter is not involved. There remains the opener inhibitor, which in *Panulirus* does inhibit the main flexor, but which there does not cross to the thicker bundle. In order to decide whether 3 or 4 separate inhibitors are present it would be necessary to use the whole leg, a procedure which would kill the animal, and would even then, because of the inclusion of the place of autotomy, be difficult to perform. Because of the scarcity of the material this type of experiment was not tried.

ZUSAMMENFASSUNG

Die Innervation der Beinmuskeln von *Homarus vulgaris* wurde untersucht. Sie stimmt mit derjenigen von *Astacus* und *Cambarus*, soweit diese bekannt waren, überein. Das Schema der motorischen Innervation ist dasselbe wie in anderen Dekapoden. Das Schema der hemmenden Fasern ist von den der anderen verschieden.

With great pleasure the author likes to express his thanks for the hospitality and coöperation received from the staff of the Zoological Station during his stay. In particular to the director, Dr. J. VERWEY, who did everything to make these months very pleasant indeed and to the secretary Miss T. STOLL. For their great kindness and willingness in lending valuable apparatus the author is indebted to professor Dr. G. P. BAERENDS, Groningen, and to professor Dr. S. DIJKGRAAF, Utrecht.

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DISCRIMINATION OF MUSICAL TEMPI BY A YOUNG CHIMPANZEE

BY

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In a former publication (BIERENS DE HAAN, 1949) I described how I succeeded, not without difficulty, in training a young chimpanzee to attend to the acoustic stimulus produced by the ticking of a metronome.

The set-up of those (and of the present) experiments was as follows. Two wooden slides, 110 cm long and 22 cm wide, were placed against the bars outside the cage of the animal. A wooden rail, 2 cm wide, was fixed at either of these slides, leaving a free space of 18 cm between them. In this space a small board could slide, to which a box was fitted, with a base of 20×24 cm, and 30 cm high. This box was divided into two parts: a lower one, 25 cm high, containing a built-in metronome, and an upper one, 5 cm high, which could be opened by lifting a lid, that projected 1 cm from the box. In this upper part some food could be put, which the animal could take out by lifting the lid. The metronome in the lower part could be set ticking at a slower or quicker rate, or left silent. Fig. 1 and 2 will make all this clear. To the board below the boxes a rope was attached whose loose end was laid into the cage, so that the animal could easily draw the box towards him. The boxes were placed at the end of the slides, about 90 cm from the bars of the cage.

Before each trial a piece of apple was put in the box which, in that trial, was to be the positive one. This was done behind an iron wall of the cage and thus invisible to the animal. The metronomes then were set ticking at different speeds (in the preliminary experiments one was set ticking and the other left silent) and the boxes placed on the slides. The animal was now free to draw in one of the boxes. In order not to influence the animal's choice by the sequence in which the boxes were placed on the slides the L-(left)-box was as a rule placed first. The place of the positive box (with the fruit) was changed in the following

order: L - L - R - L - R - L - R - R - L - R (R and L as seen from the side of the experimenter); a sequence too complicated for the animal to grasp, and in which, in a series of 10 trials, the positive box was placed as often on the R- as on the L-side. In order to prevent his getting trained to choose one particular box (as he did for some time in the former experiments) I changed

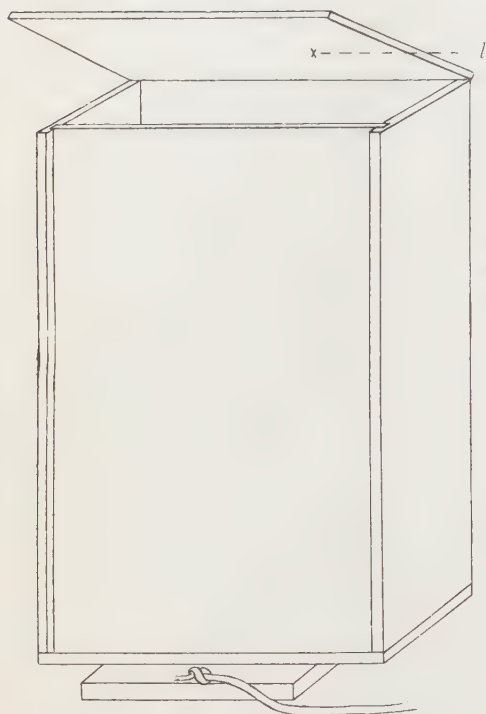


Fig. 1. One of the boxes with the lid (l) partly opened

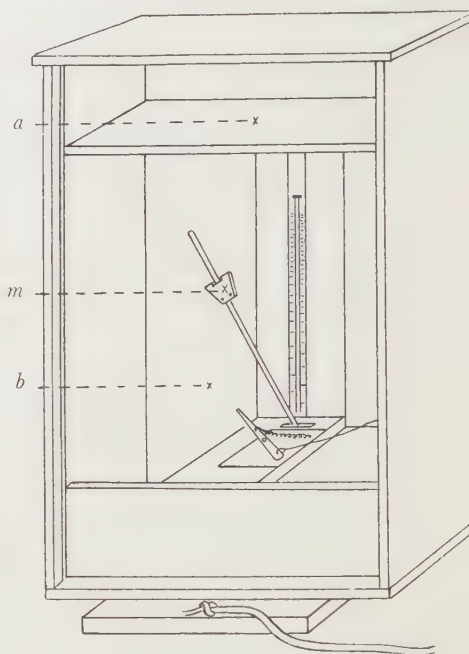


Fig. 2. Box with the front wall removed, so that the pendulum of the metronome (*m*) is visible. *a*: upper part of the box where the fruit is laid; *b*: lower part with metronome

the box which was to be chosen as the positive one at half-time during the experiments on each day.

In the preliminary experiments, described in the publication mentioned above, my task had been to train the animal to attend to the ticking of the metronome. To this end the metronome of one box was left silent, while that in the other box was set ticking at a high speed. It took me more than 1500 trials to train the

animal to choose the box with the ticking metronome ¹⁾). When I had got so far I could begin with my discrimination experiments.

As the reader will know, a normal metronome distinguishes six different tempi, viz.:

<i>Largo</i> :	42—	69	ticks per minute		
<i>Larghetto</i> :	69—	96	„	„	„
<i>Adagio</i> :	96—	125	„	„	„
<i>Andante</i> :	125—	150	„	„	„
<i>Allegro</i> :	150—	175	„	„	„
<i>Presto</i> :	175—	210	„	„	„

As my aim was to test the animal's discrimination of the different tempi, it goes without saying that I first had to examine that between the two extreme ones: *presto* and *largo*. For both an average number of ticks was chosen: about 195 for *presto* and about 55 per minute for *largo*. The choice of the *presto*-box was rewarded.

After one control series, in which I let him choose once more between a ticking and a silent metronome (in which in a series of 10 trials he made no error) the results in series of 10 trials were:

1st day:	4, 5, 6, 8	right choices (58%)
2d „ :	6, 8, 4, 8, 7	„ „ (66%)
3d „ :	7, 9, 7, 8, 9	„ „ (80%)
4th „ :	8, 6, 8, 9, 9	„ „ (80%)
5th „ :	8, 9, 8, 9, 9	„ „ (86%)
6th „ :	9, 10, 7, 9, 9	„ „ (88%)
7th „ :	9, 9, 9, 9, 8	„ „ (88%)
8th „ :	8, 10, 9, 10, 10	„ „ (94%)
9th „ :	10, 10, 10, 10, 9	„ „ (98%)

By the results of these 440 trials the discrimination between *presto* and *largo* could be regarded as sufficiently proved. I now passed on to the discrimination between the following tempi: *allegro* (about 165) and *larghetto* (about 72 ticks a minute).

The discrimination between *allegro* and *larghetto* after the previous training proved to be very easy for him. It was as if he did not hear much difference from the former tempi. The first day his right choices were: 8, 9, 10, 9, 10 (92%), the second day 9,

¹⁾ Another and evidently more intelligent, or more attentive, young chimpanzee with whom I afterwards repeated these experiments learned the discrimination between the silent and the ticking metronome in about 200 trials.

10, 10, 10, 10 (98%) in five series. Only a few times did I see him listening attentively, as if the choice was somewhat difficult for him.

It was to be expected that the discrimination between *andante* (about 138 ticks) and *adagio* (about 108 ticks) would give him more trouble. Even for myself this discrimination was not easy. I had to listen attentively to the ticking of the metronomes before I was sure which was the quicker one. It appeared that for him, too, it was at first chiefly a matter of attention if he drew in the box with the more quickly ticking metronome. Several times I saw him hesitating before making a definite choice: he then listened sharply for some moments, holding his head on one side. And even then he was not always sure of himself. Several times he drew in one box for some distance, listened again, threw away the rope, took the other rope and drew in the other box, sometimes changing his mind as often as four times before he made his final choice, which now ended in the right choice, now in the wrong one. Yet his choices improved fairly soon and it became clear that he learned to distinguish also between these two tempi. The results of his choices were:

1st day:	7, 6, 7, 7	right choices (67%)
2d	„ : 9, 10, 9, 8, 8	„ „ (88%)
3d	„ : 9, 10, 9, 8, 9	„ „ (90%)
4th	„ : 9, 10, 9, 9, 9	„ „ (92%)
5th	„ : 10, 9, 10, 9, 10	„ „ (96%)

With this result I regarded his discrimination between *andante* and *adagio* too as sufficiently proved.

He could distinguish, therefore, between 138 and 108 ticks a minute. Could he go farther and distinguish between speeds closer together? I first tried the discrimination between 132 and 114 ticks, in which the choice of the box with 132 ticks was rewarded. This proved to be too difficult for him. The first day his hesitations increased: in two trials he changed his choice as many as 8 times before making a final choice. The results of the first day were 62% right choices; on the second day 60% right choices. By this results, and by his behaviour at the moment of choice, it was clear that there was no reason to continue this test. All the same, the following days I gave him the choice between 126 and 120 ticks a minute, to see what would happen now. As might have been foreseen, this discrimination was completely

beyond his capacity. He did no more listen very much, nor even show his former hesitations. He began to choose haphazardly, chose very quickly, certainly more quickly than the time I wanted myself to hear a difference between both speeds. His uncertainty was shown also by his choices: the first day he chose 41 times (82%) the L-box; the second day 38 times (76%). In other words: where discrimination failed, he fell back on a more primitive way of choosing, viz. the choosing of the box in a particular place (*in casu* the left one) instead of the box which was distinguished by the quicker ticking metronome. Anyhow it is evident that the limit of his discrimination lays at about the same difference of rate of the ticking as in man.

As far as I know similar experiments have been performed with animals only three times, be it partly for other purposes. According to PAVLOV (1928) one of his collaborators found in the dog, with his method of the conditioned reflexes, a discrimination between 100 and 96 ticks a minute; a discrimination much finer than that of man, and also much finer than that of my chimpanzee. In how far his method was reliable may be left out of discussion. ANDERSON and LIDDEL (1935) used a similar method with sheep. After letting a metronome tick during 3—10 seconds at the rate of 120 ticks a minute an electric shock was administered which caused the animal to retract a leg. When this conditioned reflex was fixed they studied the effect of slower tickings, after which no shock was given. Only with one sheep, a castrated male, they found a discrimination; the other two animals fell into a state of neurosis which made working with them impossible. The first animal proved to distinguish a rate of 120 ticks a minute from 50, 60, 72, 84 and 92 ticks respectively, but failed to distinguish 120 from 100 ticks a minute. FRENCH (1942) trained 38 rats to jump on to a platform in their cages to evade an electric shock when the clicks of a clockwork were presented with a rate of 5 per second (i.e. 300 a minute) and to stay at the bottom of the cage during tickings at the rate of one click per second. The training was regarded as successful when in 53 out of 60 trials in two successive days (87%) the right response was evoked. This was learned by the rats in 9—25 days (average 15.6 days). After this the discrimination between faster tickings and the original rate was studied. FRENCH does not give any figures, but from his curves it may be concluded that a 80% right distinction occurred between 3 and 5 ticks per second (180—300 a minute). It seems

questionable, however, whether 5 ticks a second are still perceived as separate sounds by the animal.

It may be asked what is the use of all this, and why it is important to know if an animal distinguishes between different speeds of ticking of a metronome. This problem is, however, related to that of the animal's *knowledge of time*. Some philosophers (f.i. NIETZSCHE) have contended that the animal lives outside of time; that it does not know time. Later experience has proved that this contention is erroneous. First of all, already the first experiments with animals in mazes have shown that at a point in the maze where two ways were open to the goal, the animals learned fairly quickly to choose the shorter of the two. This preference for a shorter out of two ways must be based on an experience of the duration of the time necessary to reach the goal along each path respectively. Further, ANDERSON's (1932) rats were confined for one, two, three or four minutes on their way to the food, according as they chose the first, second, third or fourth out of four passages to it. Whereas, at first, the rats chose, on an average, 21, 20, 23 and 35 times in 100 trials the passages in which they were held up for 1, 2, 3 and 4 minutes respectively, in the last of a series of 100 days they did it on an average 72, 19, 6 and 3 times respectively. This proves that they distinguished quite well between these four durations, and conceivably preferred the shortest delay. To quote also a more recent result: HERON (1949) found with a similar retention method that rats could distinguish between a retention of 5 and 45 seconds, of 5 and 35 seconds, and of 5 and 25 seconds, while one of his animals even distinguished between 5 and 10 seconds. BELING's and others' experiments on the perception of time-durations in bees and ants (see VON STEIN-BELING, 1935) are too well-known to require detailed statement here.

That animals experience time durations is now, therefore, without any doubt. But we may ask: does the animal experience this duration of time as one continuous flow of it, having only the quality of length, or may its experience of duration be a structural one? In other words: is the animal able to perceive *rhythm* and *tempo* in the flow of time it experiences? In regard to the first question several experiments have been made. I would only remind the reader here of the rhythmical alternations of CARR's (1917) rats; the experiments of HUNTER (1920, 1928) and

others in the "temporal maze", and my own experiments with rhythmical action induced in monkeys (1935, 1936). On the problem of discrimination between different tempi, not many experiments have yet been performed; they are described above. Yet this problem of the discrimination between different velocities is also of importance for our knowledge of time-experience in animals. For if an animal distinguishes between two different speeds of a metronome, this distinction must be based on the experience of a duration of time, whether the animal distinguishes between the numbers of ticks during the same duration of time, or between the duration of the different intervals between two successive ticks of the instrument. That no explicit counting is required in such discrimination, and certainly is not involved in it in the case of the animals, may be said in parentheses.

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THE THREE SPECIES OF *TROGONOTHERIUM*, WITH A REMARK ON *ANCHITHERIOMYS*

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Since the monograph on *Trogontherium* (SCHREUDER, 1929) appeared, followed by an abstract in the *Palaeontologische Zeitschrift* (1931), some publications on Castoridae have seen the light¹). They render it desirable to give a short recapitulation of the data known at present respecting the three species of *Trogontherium*, followed by a note on the desirability of uniting the genera *Anchitheriomys* (ROGER, 1898) and *Amblycastor* (MATTHEW, 1918) to one genus, viz. *Anchitheriomys*.

Trogontherium minus Newton.

1890. *Trogontherium minus*, NEWTON, p. 447, Pl. 18, figs. 5, 6; Felixstow, max; fragm. with p⁴ — m².
1891. ———, NEWTON, p. 51, Pl. 5, figs. 17, 18; *ibid.*, *idem*.
1890. *Castor spec.*, DEPÉRET, p. 47, Pl. 4, fig. 17; Perpignan, fragm. of femur.
1897. *Castor praefiber?*, DEPÉRET, p. 197; *ibid.*, *idem*.
1927. *Trogontherium minus*, SCHREUDER, p. 375, fig. 1; *ibid.* *idem*.
1936. *Steneofibr?* *spec.*, WINOGRADOFF, p. 96, Pl. 1, figs. 4, 5, textfs. 1, 2. Selim-Djevar (N. Kazakhstan = Kirgistan, USSR, Asia), mand. with teeth, 4 isolated upper teeth.
936. *Trogontherium cuvieri?*, WINOGRADOFF, p. 95, Pl. 2, figs. 1-3; *ibid.*, 3 frags. of femora, 1 of hum.

This smallest and, geologically, oldest species of *Trogontherium* NEWTON based on a maxillary fragment with teeth out of the lowest strata (Nodule Bed) of the Red Crag. In 1890 as locality Woodbridge was mistakenly mentioned; afterwards it was corrected into Felixstow (NEWTON, 1891, p. 51, foot-note).

The width of the premolar in the English type jaw is 8 mm. In the many specimens of p⁴ of *Trogontherium boisvilletti* from Tegelen the width is 12 mm on an average; the ratio is thus about 2 : 3. The same ratio is shown by the length of the femora, viz. ± 70 mm in the specimen termed *Castor spec.* described and

¹, I regret having tried in vain to take cognizance of TEILHARD's publication (1942) on pliocene New Castorids from North China.

figured by DEPÉRET (1890) out of the Astian fauna from Perpignan, Roussillon (SCHREUDER, 1927), and 105 mm on an average on several femora from Tegelen.

The small species has also been found in Asia, as in 1936 WINOGRADOFF gave a description of some remains of rodents gathered by ORLOV in the province of Almolinsk, in the republic of Kazakhstan (named Kirgistan prior to 1925). Unfortunately, these remains did lie at a secondary place, and in consequence were fragmentary and of different geological age. The smallest of the three castorid species described and figured by WINOGRADOFF is represented by a fragment of the mandible with $m_1 - m_3$, three incomplete femora, the distal half of a humerus and most probably by four isolated upper teeth of which only the premolar has been figured (Pl. I, fig. 5).

The length of the best preserved femur must have been 75 mm. The Russian author very properly observed that the bone agrees to the smallest details in form with the femora from Tegelen, but is much smaller, and this is also the case as regards the humerus with the characteristic epicondylar foramen. WINOGRADOFF evidently did not know the small species established by NEWTON; so he wrote: "the question as to whether these bones belong to *Trogontherium cuvieri* or not, must provisionally remain unsolved".

Without any doubt the mandibular fragment recorded by the same author to *Steneofiber?* spec. and found at the same locality, must likewise be referred to *Trogontherium minus* Newton. Just like the limb bones mentioned above it is strongly fossilized ("clanging"), and very dark, almost black. The premolar is lacking, the three molars are preserved. The length of the alveolar row is 28-30 mm. In the Tegelen species this measurement is 42-43 mm at mandibles with teeth in the same stage of wear; thus again a ratio of 2 : 3. According to the Russian author, the length of the crown surface of p^4 and of m^3 is considerably greater than that of both the other molars. Moreover, the secondary folds on the crown surface have been isolated to two islets. These characters are those marking *Trogontherium*, and thus it is sufficiently sure that the "remains of an interesting small castorid similar to some primitive and geologically older representatives of the genus *Steneofiber*" must be referred to *Trogontherium minus*. The femora and the humeri of *Steneofiber* (SCHREUDER, 1928, figs. 2-4, 7, 8) have another form.

Although no other remains of the small species of *Trogotherium* have been recorded than those mentioned above, the bones saved prove that the habitus and the manner of living must have been the same as in the larger species from Tegelen of which the whole skeleton is known. The body of the latter was of beaver size, that of the small species will have been the size of the beaverrat (*Myocastor coypus*).

DEPÉRET (1923, p. 57) considered the English Red Crag to be the base of the Villafranchian. Its lower portion, the Nodule Bed, lies beneath the Walton Crag which according to TESCH (1934) is synchronous with the Belgian-Dutch Scaldisian, and is now considered to be Upper Pliocene (OAKLEY, 1949). The first glacial stage (Günz I) left its traces in England in the Newer Red Crag (ZEUNER, 1937), thus after the forming of the Waltonian Crag. PILGRIM (1944) tries to justify the classification of the Red Crag (which he considers as a whole) in the Pleistocene, but the fossil mammals of the Nodule Bed are synchronous with those of the Astian fauna of Roussillon (DEPÉRET, 1893, p. 307) which is undoubtedly Pliocene. SCHAUB (1943, p. 86) considers the fauna of Roussillon as early as the Lower Pliocene and refers to DEPÉRET (1890); and PILGRIM (1944, p. 31) wrote: Astian (Middle Pliocene). In the Nodule Bed as well as in the Astian fauna of Roussillon have been found remains of *Trogotherium minus* which in this way is the pliocene forerunner of both pleistocene species which are considerably larger.

It must be regretted that OSBORN, who (1922, p. 439) wrote that the Norwich Crag presents a distinct advance over the Red Crag in its mammalian as well as in its molluscan fauna, did not take over the interrogation mark which NEWTON (1890, list on p. 116) placed after *Trogotherium minus* in his list of mammals from the Norwich Crag. So it could happen that ZEUNER (1945, p. 259) mentioned the species *minus*, together with *cuvieri*, among the mammals of the Norwich Crag. Yet NEWTON's provisional record of *minus* from the Norwich Crag (1890, p. 447) was based on an incisor (OWEN, 1846, p. 192, Pl. 18, fig. 6) which might as well be a young incisor of *Trogotherium boisvilletti* of which still smaller young incisors have been found in the Tegelen clay (SCHREUDER, 1929, Pl. 6, figs. 10 and 13). I cannot agree with ZEUNER (1945, p. 258) who considers *Trogotherium* to be a new-comer in the Lower-Pleistocene of Europe. *Trogotherium minus* is pliocene as we saw and *Trogotherium boisvilletti* occurs in

the fauna of Chagny (COLLOT, 1906), a Villafranchian fauna older than that of Senèze which is placed by ZEUNER before the Lower-Pleistocene. In my opinion it must have been in the long period between the Nodule Bed of the Red Crag and the Norwich Crag in England, and between the Astian of Roussillon and the Villafranchian of Chagny in France that *Trogontherium minus* was replaced by the considerably larger *Trogontherium boisvilletti* in Western Europe.

The age of the Russian specimens cannot be made out, but the high degree of fossilization betrays that also in Asia the small species of *Trogontherium* was older than the Pleistocene. PEI (1939) in his geochronological Table quite rightly mentions *Trogontherium* among the pliocene survivals, but, unfortunately he takes (p. 5) the species *cuvieri* as such, the species which is geologically youngest and does not occur before the Günz-Mindel Interglacial and still lived in the Mindel II glaciation, f.i. in Süssenborn (SOERGEL, 1939).

Trogontherium boisvilletti (Laugel)

1840. Species of Beaver, LYELL, p. 362; Forest Bed, mand.
 1846. *Trogontherium Cuvieri*, OWEN, p. 184, figs. 71-73; *ibid.* 3 mand. with teeth.
 1846. A Beaver, OWEN, p. 192; Norwich Crag, Sizewell Gap, young lower incisor.
 1848. *Diabroticus Schmerlingii*, POMEL, p. 167; OWEN's material.
 1852. Beaver (extinct? species), BROWN, p. 188, fig. 3; Copford, Essex, p⁴ and other tooth.*
 1869. *Trogontherium Cuvieri*, OWEN, p. 49, Pl. 3, textfs. 1, 3-5; Forest Bed, max. with teeth, inc. hum., femur, tib.-fib., calc.
 1862. *Conodontes boisvilletti*, LAUGEL, p. 751, 1 Pl.; Saint-Prest, post. half of skull, mt. IV.
 1867. *Trogontherium Cuvieri*, LARTET, p. 48; *ibid.*, *idem.*
 1867/9. *Conodontes Boisvillettii*, GERVAIS, p. 80, Pl. 15,** *ibid.*, *idem.*
 1885. ————, SCHLOSSER, p. 44; *ibid.*, *idem.*
 1885. Castoroid rodent, near to *Trogontherium*, LYDEKKER, p. 221, fig. 30; same tooth as BROWN (1852), termed by SCHLOSSER (1902, p. 117) *Dipoides* L.
 1881. *Trogontherium Cuvieri*, NEWTON, p. 257; Norwich Crag, Thorpe, incisor.

*) This other tooth NEWTON (1916) could not find again.

**) The caption of the Plate is: *Conodus boisvilletti*, which is undoubtedly an error.

1882. *Trogontherium Cuvieri*, NEWTON, p. 65, Pl. 11; Forest Bed, OWEN's material.
1891. ———, NEWTON, p. 51; *ibid.* and Weybourn and Norwich Crag.
1892. ———, NEWTON, p. 165, Pl. 19; Forest Bed of East-Runton, skull.
1893. *Castor Issiodorensis*, DEPÉRET, p. 232, Pl. 11, figs. 6-8, and COLLOT (1906), p. 8, fig. 1; Chagny, mand. with p₄—m₂.
1893. *Trogontherium Cuvieri*, DEPÉRET, p. 253, Pl. 14, fig. 6; Saint-Cosme, mt. II.
1902. *Dipoides Lydekkeri* n. sp., SCHLOSSER, p. 117; tooth figured by BROWN (1852) and by LYDEKKER (1885).
1902. *Trogontherium Cuvieri*, NEWTON, p. 387, 3 textfigs.; Ingress Vale, incisor inf.
1903. ———, STOPES, p. 804; Swanscombe*, name in list.
1904. ———, DUBOIS, p. 248; Tegelen, name in list.
1906. ———, COLLOT, in separate p. 5, Pl. 1, figs. 2, 3; Curtil, mand. with i, femur, p. 11, fig. 4; Bligny s. Beaune, femur.
1914. ———, HINTON, p. 189, Pl. 8, figs. 4-7; Forest Bed, 3 young incisors, navicular.
1916. ———, NEWTON, p. 322, 4 textfigs.; Copford, Essex, tooth figured by BROWN and by LYDEKKER.
1928. "*Conodontes Boisvilleti*", RÜGER, p. 33.
1938. *Trogontherium cuvieri*, OAKLEY, p. 57; Swanscombe, in list of mammals.
1938. *Conodontes (Trogontherium) Boisvillettei*, VIRET, p. 520; Bruges, p₄, fragm. of incisors.
1945. *Trogontherium boisvilletti*, SCHREUDER, p. 165, textf. 1; Tegelen.
1949. ———, SCHREUDER, p. 119, figs. 6-11; Tegelen, three more or less complete casts of skulls, endocranial cast and skull with lower jaw.

*) I can not follow ZEUNER (1945, p. 115, foot-note) who wrote: "*Trogontherium cuvieri* not found at Swanscombe, but at Ingress Vale (STOPES, 1904, p. 804). This was first pointed out by OAKLEY in the Swanscombe Report, p. 57." However, OAKLEY (1938, p. 57) wrote: "The fauna of the Lower Gravel includes *Trogontherium cuvieri*", etc. and in a foot-note: "See STOPES, p. 804. The gravels in the Ingress Vale pit from which this species is recorded are equivalent to the Lower Gravel of the Barnfield Pit."

MARSTON (1950), however, relying on KENNARD's investigation (1942) of the Mollusca, considers the Ingress Vale stage to correspond to the late stage of the infilling of Middle Gravel Erosion stage, and definitely later than the Barnfield Lower (and Lower Middle) Gravels.

This geologically younger and much larger species is the best known, because the Tegelen clay yielded practically all the skeleton bones, mostly in several specimens. Undoubtedly a colony of these aquatic animals has existed at this place. However,



Fig. 1. *Trogontherium boisvilletti* (Laugel), Tegelen. Skull with lower jaw, side view; $2/3 \times$ nat. size. Coll. Leiden State Geol. Museum.

a skull has long been a desideratum, until recently there have been found in an unused cabinet in Teylers Museum among some other Tegelen fossils described by HOOIJER (1947), two concretions which each enclose a large portion of a skull. Moreover, a very remarkable concretion could be added to the Tegelen collection in the Leiden Geological Museum, enclosing the cast of a nearly complete skull with a very fine endocranial cast within. And past year the same collection was enriched with a fairly complete skull (the jugal arches, and the base with the posterior wall of the braincase are lacking) together with the lower jaw. The finder, Mr. G. KORTENBOUT VAN DER SLUIJS, assistant at the Rijksmuseum van Geologie at Leiden collected in the clay the many pieces in which the bones were broken and succeeded in making a satisfactory restoration (fig. 1).

The smaller concretion in Teylers Museum (15795) shows the hinder portion of a skull which has been broken off through the fossa olfactoria and ventrally through the ductus naso-pharyngeus just in front of the teethrows. The bones of the occiput are almost intact, but all teeth are lacking. The absence of the bones of the skullroof reveals the upper surface of the endocranial cast (fig. a of Pl. XX). The hemispheres have a joint width of 52 mm and a length of about 50 mm. In the anterior portion of each of them a shallow dint can be observed close to the median line. The transversal groove separating cerebrum and cerebellum is situated so near to the posterior end of the skull, that the antero-posterior diameter of the cerebellum is not more than 6 mm.

Width and height of the occiput are 83.7 and 40.8 mm. This is the only specimen from Tegelen in which the posterior wall of the skull, the condyles, the basioccipetal, and the adjacent petro-tympanicum of both sides are present in situ. In the French type skull the width is just the same, but the height is 46 mm there, caused by the presence of the rather high occipetal crest which has got lost in the Tegelen object.

The endocast of the ductus naso-pharyngeus is present along the base of the anterior portion of the object. Its height is 22.5 mm, and the width (between the roots of the molars) is 12 mm. Ventrally it shows the fine median groove corresponding to the thin median crest on the dorsal surface of the maxillary.

Of the larger concretion (15794) in Teylers Museum, on the contrary, the occiput is lacking, the skull (Pl. XXI) being broken off behind the zygomatic arches and the teeth rows. Thus the

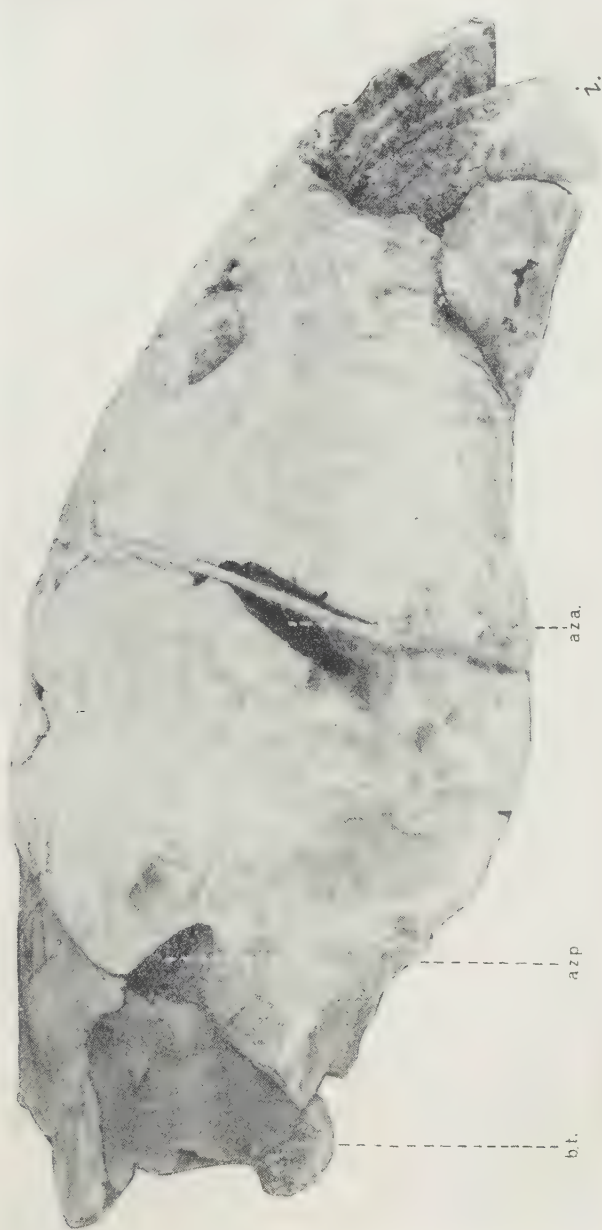


Fig. 2. Concretion enclosing cast of skull of *Trogontherium* (Lauget), Tegel. View of right side; $\frac{3}{4}$ nat. size. Coll. State Geol. Mus., Leiden St. 47497. b.t. bulla tympani; a.z.p. post. place of origin of arcus zygomaticus; a.z.a. ant. place of origin of the latter; i. cast of alveole of the right incisor.

hinder portion of the braincase is missing, and so is the dentition with the exception of the greater part of the left incisor, and the premolar with the defect first molar of the right side. These grinders show that we have to do with a fullgrown skull, all the enamel folds being isolated to islets on the grinding surface. All the bones of the skull are in situ, although broken to pieces (fig. a) and the sutures are dislocated, due to the expansion of the substance filling the skull. The colour of both Teyler specimens is reddish brown, caused by iron oxyde.

I could convince myself of the identity of the Tegelen and the French (type) skull by the fine plastercast of the latter I received several years ago from Professor Boule. Moreover, the Dutch skull neither shows any difference with the English skull described and figured by NEWTON (1892), except for the somewhat larger size of the latter, in accordance with its older age.

Comparing the Leiden cast of skull (St. 47497) with No. 15794 in the Teyler collection the most salient difference is the extraordinary size of the former. The length of the Leiden specimen, from the middle of the interparietal bone to the anterior point of the rostrum is 215 mm, against 160 mm in the Teyler skull. The relation in size is about 4 : 3 and we meet it in all other measurements of the casts. For instance Pl. XXI, fig. b. shows the Teyler skull at $\frac{3}{4}$ natural size, but the same view of the Leiden specimen (Pl. XXII, fig. 4b) has been taken little more than $\frac{1}{2}$ nat. size, and yet the figures are about equal in size.

The concretion in the Leiden Museum has the grey colour of the clay in which it was inbedded. It is a lump of stone (fig. 2), about 23 cm long and 10 cm in diameter. The surface is rather smooth and only pierced by two pairs of slots. The anterior pair stands vertically and the posterior pair is horizontal. They mark the ends of the zygomatic arches and are caused by the crumbling away of the latter and proof that the arch was still present when the concretion was formed. An x-ray-foto has been taken of the object on the initiative of Dr. D. A. HOOIJER, at Leiden, in order to detect the contents of it. It revealed the presence of the cast of an almost complete skull of *Trogontherium*. The stony casts of the large holes are indicated by dark spots on the x-foto. The light places indicate where the bones of the skull were present, before they crumbled away and left open spaces. Guided by the x-ray foto's it was possible to open the concretion without doing

much harm to the cast within. Along an artificial sagittal burst the right wall can be removed now and then the greater part of the cast lies open to the eye (Pl. XXII). The cast of the ductus nasopharyngeus is slightly S-shaped, which is the effect of the deformation of the skull by three transversal fractures. The foremost of these runs through the dislocated maxillary-premaxillary suture and the casts of the incisors and is the cause that the latter do not have the normal form of a circle-segment, but are stretched out in the length (fig. a). The second fracture runs just behind the end of the alveoles (*b.a.*) and through the suture between the presphenoid and the ethmoid. The hindermost fracture separates the occiput from the sphenoidal portion of the skull. The fractures appear to have been consolidated afterwards so that the cast of the skull is one firm stony object, and so is the crust with which it is united. On fig. a the crust can be seen all along the upper surface of the cast and ventrally at the incisor foramina. Innumerable cracks pierce the surface of the crust as well as that of the cast. Most of them appear to have been filled up afterwards. This process must have taken place repeatedly and has caused the remarkable expansion of the object and its abnormally large size.

At the ventral side (fig. b) between the (casts of) alveoles the (cast of the) ductus naso-pharyngeus can be observed from the begin almost to the end, the posterior portion is lacking. In the larger Teyler specimen it is still in place although the same ruptures can be seen in both objects, causing the S-shaped form of the ductus. No trace of bone *in situ* nor of the teeth can be detected in the Leiden cast. Some bone-dust may occur in the spaces between the casts and in the dislocated sutures. The cast of the ductus naso-palatinus (= incisivus) is intact at both sides. It runs like a thick rope from the upper posterior end of the nasal chamber vertically downwards round the cast of the infra-orbital foramen and then round the cast of the alveole of the incisor to the incisive foramina in which it ends at the dislocated premaxillary-maxillary suture. Also in HELLER's figures of the beaver skull cast from Mauer part of this ductus is very distinct.

At the left side of the skull the crust is ventrally connected to the end of the cast of the ductus naso-pharyngeus (fig. b). Behind this connection the inner side of the dorsal crust bears the impressions of the decayed skull bones (frontalia, parietalia, interparietale).

Seen from the side (fig. a) the dislocation of the cast of the large alveole is apparent and so is the crack through the ductus naso-pharyngeus behind the bottom of the alveole. Posterior to this crack and above the ductus naso-pharyngeus the cast can be observed of the large transverse foramen in the praesphenoid. The cast of the right tympanic bulla is present in situ, and so is the cast of the meatus auditivus externus. The concretion is open posteriorly and thus no impressions of the occipital bones can be observed, nor of the condyles.

The endocranial cast was connected to the crust merely by the casts of the canals and the foramina which penetrated the lost skull-bones. When the concretion was opened these brittle connections break off as a matter of course and in this way the endocranial cast could be taken out of the concretion and can be studied from all sides (Pl. XXIII). This object also is penetrated by numerous cracks, especially the posterior half. The size is much greater again than the Teyler specimen (15795) described above in which length and width of the cerebrum (hemispheres) are 50 and 52 mm respectively, whereas in the Leiden endocast these measurements are 60 and 63.6 mm.

In describing endocranial casts it is usage to use terms as if the brain itself had been fossilized. However it must be held in mind that we lack data concerning the thickness of the circum-cerebral space (meninges, bloodvessels and cerebro-spinal fluid) between the brain and the cranium. By the presence of this space sulci are less distinct on the inner surface of the cranial bones than they are on the brain itself.

The only figures of the brains of *Castor* I met with in literature are those given by LEURET et GRATIOLET (1839/'57, Pl. XXII, figs. 1 and 2) and referred to by BEDDARD (1892, p. 597). However, the brains figured must have been badly damaged, as no trace of the olfactory bulbs has been drawn, nor do the authors mention them, although they are large (BEDDARD) and situated entirely anterior to the hemispheres in a somewhat separate compartment of the braincase. Therefore we must assume that the bulbs must have broken off when the brain was taken out of the skull. According to BEDDARD the length of the hemispheres in *Castor canadensis* is 38 mm and their united width is 41 mm. HELLER (1939) measured at the endocranial cast of the pleistocene Mauer Beaver a length of 46 and a combined width of 53 mm, which is in accordance with the larger size of the latter.

At the endocranial casts of *Trogotherium* from Tegelen the corresponding numbers are as we saw 50 and 52 mm in the Teyler specimen and 60 and 63 mm in the Leiden object.

Fossil brains of Rodents are very scarce and as regards Castoridae the only description I know is that of *Castor fiber* from MAUER, just mentioned. This concretion has much in common with the larger concretions of *Trogotherium* in Teyler Museum as regards the presence of some grinders and of matrix concealing the ventral side of the brain.

According to BURNE and ELLIOT SMITH (1902) the most noteworthy features of the brain of the Beaver are the obliteration of a great part of the rhinal fissure and the utter absence of the sulci in the large pallium. "This is one of the enigmas of cerebral morphology which we are utterly unable to explain satisfactorily at present. The smoothness of such large hemispheres in the brain of the beaver is a peculiar phenomenon which is much rarer than it is generally supposed." (p. 195).

There is a shallow sagittal furrow or dint (HELLER's sagittale Längsfurche) on the dorsal surface of each hemisphere, which might represent either the corono-lateral or the suprasylvian sulcus. BEDDARD (1892, p. 610) terms it an "obscure dint" and mentions too the entire absence of convolutions.

Likewise in *Trogotherium* there cannot be detected any sulcus in the pallium and the sagittal furrow is here a shallow round dint. In the Teyler specimen some thin parallel bloodvessels run forward from the posterior margin of the hemispheres (Pl. XX, fig. a). In the Leiden cast the arteria meningeal is distinct. (Pl. XXIII, fig. c).

Just before the dints mentioned the impression of the dislocated sutura coronalis between the parietalia and the frontalia is observable on the hemispheres. Its light colour is caused by the bone stuff in the suture. For the same reason the other sutures between the skull bones are distinct.

Behind the large hemispheres the cerebellum has a rather short antero-posterior extent, not more than about one centimeter. The large olfactory bulbs with their pedunculus project entirely anterior to the hemispheres and are situated so closely against each other that only at the ventral side a median fissure in the cast is present between both bulbs, not between the peduncles. From the olfactory bulbs forwards some thin casts of the filii olfactorii project, which all have been broken off at the level of the lost lamina cribrosa.

The ventral side of the brains (Pl. XXIII, fig. b) exhibits a wide transversal fracture through the foramen lacerum anterius. Along this fracture the posterior portion of the endocranial cast has shifted to the left side on the figure. This posterior part exhibits the medulla oblongata with the bifurcated cast of the porus acusticus through which runs the nervus acusticus (VIII).

NEWTON compared the English skull with the Russian skull of *Trogontherium* too, of which he had a cast at his disposal. He thought both skulls to be identic, but he added: "There is one point, however, about which I am in some doubt; in the description of FISCHER's type attention is called to a deep fossa, situated on the base of the skull between the tympanic bullae. The cast of the specimen has a deep hole in this region", etc.

Comparing the bases of the Russian, the German, the English, the French and the Dutch skull of *Trogontherium* with that of *Castoroides* (see Pl. II of the monograph of *Trogontherium*, 1929), it is clear that the deep hole mentioned of the Russian skull between the tympanic bullae, and distinctly to be seen on the figure of that skull, is absent in the English and in the French specimen. As regards the German skull fragment and the recently discovered Dutch fragments the skull portion in question has got lost.

Castoroides which possesses a similar ductus pneumaticus cranii as *Trogontherium cuvieri*, shows at the corresponding place, or perhaps slightly anterior to it, the hinder opening of that passage. So I lean to the opinion that the hole in the Russian skull is an analogue of that opening in *Castoroides*. It would be worth the trouble of clearing away the thick layer of sandy matrix which covers the base of the Russian skull. NEWTON thought it impossible that, where the Russian and the English skull agree in form up to the slightest detail, there could be so striking a difference at the base of the skulls.

This error of NEWTON's has been the reason that for half a century the West-European species *Trogontherium boisvilletti* appeared in literature with the name of *Trogontherium cuvieri*.

There is not any difference between the skull bones from Tegelen and those of the type skull from Saint-Prest. Thus the Tegelen species was termed *Conodontes boisvillettii* Laugel in the monograph (1929). I agree, however, with STIRTON (1935) that the difference between this species and the more specialized

eastern form justifies the distinction of two species indeed, but it is not great enough to allow a division of the pleistocene *Trogontherium* into two genera. Thus the generic name of *Trogontherium* (FISCHER DE WALDHEIM, 1809) must prevail over *Conodontes* (LAUGEL, 1862). Since 1936 I replaced the generic name of *Conodontes* by *Trogontherium* for the Tegelen form. I regret to have written formerly two i's at the end of the specific name, following GERVAIS, LARTET, NEWTON and several other authors when treating of the nomenclature of the French skull. The name of the West-European species must be: *Trogontherium boisvilletti* (Laugel), as I termed it since 1945.

The rich and extraordinary well preserved material from the Tegelen Clay proves that the animal was practically as large as the beaver. Only the jaws are more robust, evidently to give room to the very strong incisors. The largest series of vertebrae ever gathered in Tegelen is the sacrum consisting of 4 vertebrae, as in *Castor*. In the latter the length of the four together is 81 mm, in *Trogontherium* it is 87.5 mm. The body of the latter thus will have been little longer than that of the beaver, and it was somewhat more slender. The combined length of the proximal and the distal bone of each extremity is the same in both animals, as is apparent from the Table. The greater length of the distal, and the shortness of the proximal, bones in the extinct animal point to a greater mobility of hand and foot in *Trogontherium*, and to the fact that it stood higher on its legs.

Foreleg	<i>Castor fiber</i> recent	<i>Trogontherium</i> <i>boisv.</i> Tegelen Clay	<i>Desmana</i> <i>moschata</i> recent
1. length of humerus	92	85	23.6
2. length of radius	98	110	24
1 + 2	190	195	47.6
2 : 1	1.07	1.29	1.02
Hindleg			
1. length of femur	117	105	25
2. length of tibia-fibula	142	153	45
3. length of foot	180	220	58
1 + 2	259	258	70
2 : 1	1.21	1.46	1.8
3 : 1	1.54	2.10	2.20

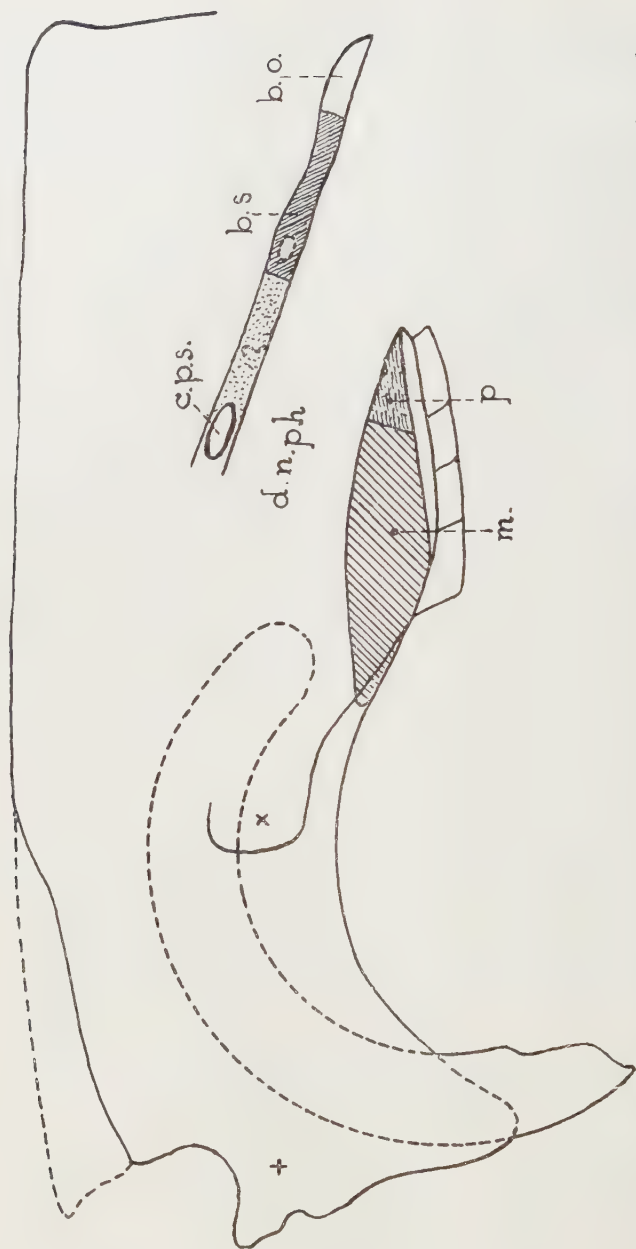


Fig. 3. *Trogontherium boissilletti* (Laugel). Reconstruction after type skull (median section); front part bones from Tegelen in side view. Crosslets indicate the bony protuberances which are the places of insertion of the strong muscles of the upperlip. c.p.s. canalis praesphenoides = can. transversus; other letters as in fig. 5. Nat. size.

The proportions of the hindleg resemble those in Watermoles. The foot is considerably larger than that of the beaver, and the tail round, probably up to the end. The mighty large webbed hindfoot and the strong tail give evidence of strong swimming power. The foot articulation is strengthened by the articulation of the calcaneum with the fibula, which does not occur in any other simplicidentate rodent. All in all, *Trogontherium* will have been still more skilful a swimmer than *Castor* and as much at home in the wet element as is the Watermole.

In front of the snout the premaxillae show (fig. 3) a broad roughened area, the upper part of which forms a pair of tubercles encroaching upon the lower margin of the nasal opening. This area has given origin to the strong muscles of the upper lips. Also the processus overlapping the suborbital foramen has given attachment to lip muscles, much stronger than those of the beaver in which no processus occurs, but a low crest. It is beyond doubt that *Trogontherium* was still more vigorous a gnawer than the beaver is, the mighty incisors having been the tools with which tree-cutting was, chiefly in winter, done. The strong upper lips justify the supposition that the animal when swimming snatched its food from the surface of the pools and river branches, especially so in summer when waterplants were abundant.

Trogontherium minus died out before the Günz glaciation. *Trogontherium boisvilletti* was very common in Tegelen and lived there in the Günz Interstadial together with Villafranchian animals like the archaic form of *Archidiskodon meridionalis*, *Sus strozzi*, *Leptobos*, etc. (SCHREUDER, 1945) in the Lower-Pleistocene. In Chagny the species was accompanied by *Anancus arvernensis* and *Archidiskodon planifrons*, *Tapirus*, *Gazella burgundina*, etc. (MAYET et ROMAN, 1923). Thus it made out part of the Upper- as well as of the Lower-Villafranchian fauna. But also later, during the Günz-Mindel Interglacial, in the Forest Bed of East-England many remains were deposited together with those of *Hippopotamus*, *Cervus elaphus*, *Bison*, etc. VIRET (1938) recorded some teeth from Bruges near Bordeaux, found together with remains of *Elephas antiquus*. The occurrence at Swanscombe would furnish evidence that in England *Trogontherium* also outlived the Mindel glaciation (PEI, 1939, p. 4, foot note). The existence of this large rodent during the Villafranchian and the Lower as well as the first part of the Middle Pleistocene reduces its value as a guide fossil.



Fig. 4. Reconstruction of *Trogontherium* from Tegelen; drawn by Mr. J. F. Obbes, Voorschoten.

To the east of the Rhine valley the Tegelen species has not been met with; there the genus was represented by the somewhat stronger and more specialized *Trogontherium cuvieri*.

Trogontherium cuvieri Fischer

1809. *Trogontherium* (*Cuvieri* in MS), FISCHER DE WALDHEIM, p. 206, Taganrok, skull.
- 1824/25. *Castor Trogontherium*, CUVIER, p. 59; ibidem, idem.
1847. *Trogontherium Cuvieri*, ROUILLIER, p. 33, Pl. 5, figs. 1-5; ibidem, idem.
- 1870/75. ———, SANDBERGER, p. 827; Mosbach, name in list of mammals.
1880. ———, KOCH, p. 50; ibidem, idem.
1885. *Castor fiber*? SCHLOSSER, p. 45; skull of Taganrok.
1887. *Trogontherium Cuvieri*, ROEMER, p. 257; Mosbach, name in list.
1895. ———, ROEMER, p. 190; ibidem, some mandibles with teeth.
1898. ———, SCHROEDER, p. 223; ibidem, idem.
1902. ———, SCHLOSSER, p. 165; ibidem, idem.
1906. ———, SCHLOSSER, p. 141; Jockgrim, name.
1909. ———, FREUDENBERG, p. 67; ibidem, idem.
1911. ———, FREUDENBERG, p. 119; Weinheim, tooth (in letter, m²).
1912. ———, REICHENAU, p. 221, Pl. 3, fig. 15, Pl. 4, figs. 24-30; Mosbach, 6 incompl. mandible with teeth.
1927. ———, SCHIRMEISEN, p. 42, Pl. 3, fig. 2; Brno, Moravia, mand. fragm. with m₂ and part of m₁.
1928. *Trogontherium Soergeli*, RÜGER, p. 30, Pl. 3, figs. 5, 6; Mauer, 2 mand. fragm. with teeth.
1928. *Trogontherium cuvieri*, SCHREUDER, p. 297; ibidem, idem.
1928. *Trogontherium* cfr. *Cuvieri*, ZDANSKY, p. 52, Pl. 5, fig. 3; Choukoutien, p⁴.
1929. *Trogontherium cuvieri*, SCHREUDER, p. 267, Pl. 2, fig. 2, Pl. 3, figs. 1, 2, Pl. 4, figs. 1, 2, Pl. 5, fig. 1, Pl. 6, figs. 5, 6, 18, Pl. 7, figs. 3, 20, 22; Mosbach, 2 skull fragm. with teeth, 3 mand. fragm. with teeth, isolated incisor and lower premolar. Jockgrim, teeth, fragm. of clav., hum., rad., il., fem., tib.-fib.
1934. ———, STEHLÍK, p. 72; Brno, mand. fragm. with p₄, m₁, m₂, incisor (part).
1934. *Trogontherium* cfr. *Cuvieri*, YOUNG, p. 52, tfigs. 16-18, Pl. 4, figs. 13, 16, 17, Pl. 5, figs. 1-7; Choukoutien, 2 mand. fragm. with teeth, upper i, p⁴, 2 ex. of m³, some lower teeth. Tanghsan, mand. with teeth.

1935. *Trogotherium cuvieri*, SCHREUDER, p. 21, 3 tfigs.; Püspökfördö, Transylvania, upper i, p⁴, m³.
1936. —————, WINOGRADOFF, p. 93, Pl. 1, figs. 1-3; Selim Djevar, Kazakhstan, mand. with teeth. Mariopol, mand. with teeth.
1938. —————, ZÁZVORKA, p. 125, Pl. 6, figs. 1-4, /1 tfig.; Přezletice, Bohemia, max. fragm. with m¹ — m³, isolated m².
1939. —————, SOERGEL, p. 828; Süssenborn, remains of which a description has been promised, but, unfortunately SOERGEL died in August 1946.
1949. —————, SCHREUDER, p. 352; Süssenborn, m³ dext.

This geologically youngest, and most specialized, species was first known from Russia. More than 60 years later it was mentioned from Mosbach, where some mandibles came to light. In 1929 I was able to describe and figure a greater portion of a skull from there, thanks to the kindness of the late Professor SCHMIDTGEN, Director of the Museum of Natural History, Mainz. This skull fragment enabled me to establish the character by which the species *cuvieri* is distinguished from *boisvilletti*. Unfortunately, this object of great value, and many other pleistocene fossils, have been destroyed by a bombardment of the Museum, as Dr. WEILER, Mainz, wrote me after the war.

The fragments of limb bones from Jockgrim show that the body of the species from there has not been larger than that from Tegelen, but the jaws and teeth are considerably stronger and the lower premolar often shows an accessory infold at the anterior end, as is shown by Pls. 2 and 7 of the Monograph (1929), where maxillary, mandibles and teeth of both species have been figured side by side.

The chief character of *cuvieri*, (fig. 5) is a second air ductus in the skull, above the common ductus naso-pharyngeus. A similar situation has been recorded in *Castoroides* only (HAY, 1914). Also there the ductus opens posteriorly at the base of the skull between the tympanic bullae, just in front of the basioccipital. From there forwards the passage rises into the basisphenoid which it hollows out so deeply, that the roof of that bone is as thin as a sheet of paper, or perhaps there has been a foramen as in *Castoroides*, probably shut off against the braincase by a membrane in the

living animal. For the detailed description of the skull in both species I refer to the Monograph (1929).

Besides the presence of this ductus there is another character which *Trogontherium cuvieri* shares with *Castoroides*, viz. the contact of the inner pterygoid wings of both sides in the median plane, by which very large pterygoid muscles are enabled to originate

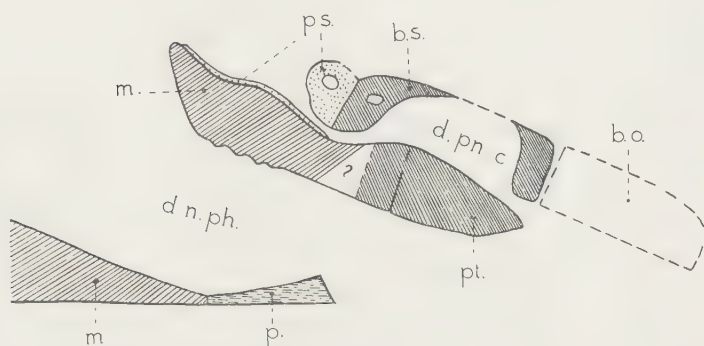
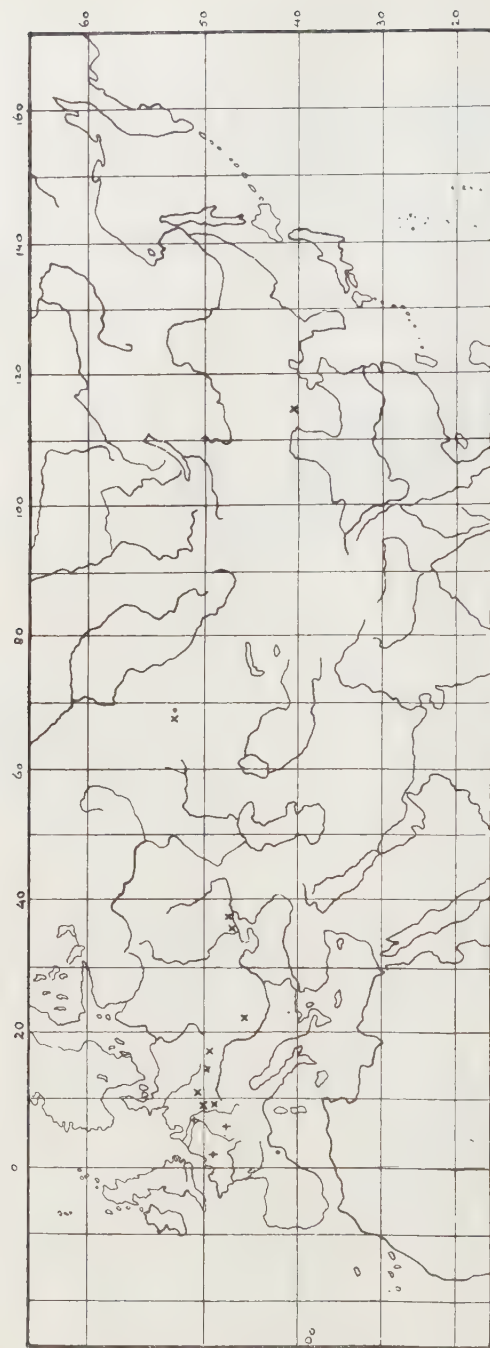


Fig. 5. *Trogontherium cuvieri* Fischer. Median section through the (lost) skull fragment from Mosbach. b.o. = basioccipital (absent); b.s. = basisphenoid; p.s. = praesphenoid; pt. = plane of contact of the inner pterygoid wings; m¹ = the same of the maxillar wings. (Whether the palatinal wings come in contact also, as is the case in *Castoroides*, could not be observed.); d.n.ph. = ductus naso-pharyngeus; d. pn. c. = ductus pneumaticus cranii; nat. size.

in the extraordinarily wide fossae. These muscles end in the round, deep fossa pterygoidea on the S-shaped plate at the base of the exceptionally strong mandible. The Mosbach skull fragment of the Mainz Museum was broken off through the large foramen praesphenoideum, so that this bone had lost its upper portion. The considerable size of that foramen in *Trogontherium* is shown by the French skull and by the cast of the Tegelen skull (Pl. XXII fig. a, *f.t.*).

The ductus pneumaticus cranii in *Trogontherium cuvieri* may have been coated with mucous membrane to produce the mucous with which the food was mixed whilst being chewed. During the Pleistocene the flora became ever poorer, and so it is not surprising to find in the youngest species, with its very strong chewing apparatus, an accommodation to soften great quantities of coarse



Distribution of the three species of *Trogontherium*.

● *Trogontherium minus*: East-Anglia, Perpignan, Siberia (Kazakhstan).

+ *Trogontherium boisvilletti*: East-Anglia, Saint-Prest, Saône et Loire (St. Cosme, Chagny, Curtil, Bligny), Gironde, Tegelen on the Meuse.

× *Trogontherium cuvieri*: Rhine-valley (Mosbach, Jockgrim, Mauer, Weinheim), Süssenborn, Bohemia, Moravia, Transylvania, South-Russia (Taganrok, Mariopol), Siberia (Kazakhstan), China (Choukoutien).

food, a specialisation not yet found in the Tegelen species which lived in more favorable times.

The large gap in the distribution of *Trogontherium cuvieri* between the locality at the Sea of Asov where the type was found by FISCHER and the *Sinanthropus* site in China has been filled by the discovery (WINOGRADOFF, 1936) by ORLOV of a mandible with teeth, together with an isolated incisor, in North-Kirgistan, to the south of Omsk. The mandible is so robust that there can be no doubt of its belonging to the species *cuvieri*. This is also the case with the isolated incisor, which is 12.3 mm broad, and thus more approaches this measurement in the Mosbach incisors which vary up to 12.7 mm, than the innumerable Tegelen specimens the width of which does not exceed 11.0 mm.

The remains of *Trogontherium* have been found by ORLOV together with those of *Trogontherium minus* mentioned above, at secondary site. They are much less fossilized than those of the small species, as their colour is light reddish brown, and they are not "clanging". Besides the adult teeth in the mandible from Kirgistan, WINOGRADOFF also figures a young one from Mariopol. Thus it is not only at Taganrok, but also at Mariopol, that remains of the large species have been found at the Sea of Asov.

Trogontherium lived together with men, in Mauer as well as in England and in Choukoutien. In Middle-Europe the genus does not appear before the Middle Cromerian (Transylvania and Moravia).

In his phylogeny of the Castoridae STIRTON (1935, chart 2) made *Trogontherium boisvilletti* the link between the small species and *cuvieri*, the latter being most specialized, not yet occurring in the Villafranchian, but common in the Cromerian of the Günz-Mindel interglacial. However, there is another possibility, viz. that the small species of the Pliocene gave origine to *boisvilletti* in the west of Europe and to *cuvieri* in the middle and the east, where (although at secondary place) the small species has been found together with *cuvieri* in Siberia, as we saw.

Which of the two large species has lived longest has not yet been made out. In 1929 neither Professor BOULE nor Dr. PONTIER, at Lumbres, ever had seen remains of *Trogontherium* from Northern-France (northern of the Seine), as they wrote me then, and so I could not mention it in the Monograph (p. 108). Afterwards BASTIN (1933) and ZEUNER (1945) mentioned *Trogontherium* from

Abbeville, like AULT DU MESNIL (1896), RUTOT (1900) and G. DUBOIS (1924) did before them. However, remains from there never have been described nor figured. Last summer Dr HOOIJER of Leiden saw in the Institut de Paléontologie Humaine in Paris a fragment of an incisor of *Trogontherium* from Abbeville, but it is not known whether this specimen came out of the "partie inférieure avec une faune comme celle de St. Prest" or out of the upper part, with human implements. (PONTIER 1928.)

Concerning the phylogenetic place of *Trogontherium* among the Beavers our knowledge there about has not proceeded satisfactorily since 1929. STIRTON (1935, p. 452) wrote: "*Monosaulax*" *eseri* possibly gave rise to these species, or it may be that *Amblycastor* with its grooved incisors was near their ancestral line. Under "*Monosaulax*" *eseri* that author understands (see also DIETRICH, 1935, p. 1076 and VIRET et MAZENOT, 1948-49, p. 52) the well-known European *Steneofiber Eseri* Schlosser (in part) and *Chalicomys Eseri* von Meyer. Now I pointed out in the Monograph (1929, p. 307) that *Steneofiber* stands more closely to *Castor* than to *Trogontherium*. And the same is the case as regards *Amblycastor*. The incisors are grooved indeed, but the limb bones are already considerably flattened, and no articulation exists between fibula and calcaneum. At least no articular facet has been mentioned of the specimen from Mongolia, nor does it exist at the specimen from Bavaria I saw. SEEMANN sees no difference with the humerus of *Castor*, so we may conclude that the epicondylar foramen, typical of *Trogontherium*, is absent. SEEMANN and STIRTON both point to the close resemblance to *Castor*. Moreover, the oldest species of *Trogontherium* known is as small as a beaverrat and lived in the Pliocene, thus later than the much larger upper miocene *Anchitheriomys* and *Amblycastor*. *Trogontherium* increases in size during the Upper-Tertiary, as is the case in so many genera of mammals. The miocene *Anchitheriomys* being so much larger than the pliocene *Trogontherium minus* at all events precludes its standing close to the ancestral line of the latter. ELMER WOOD 2nd recently (1945) recorded a lower jaw of a late miocene beaver from Montana which shows much resemblance to *Amblycastor fluminis*, although it is much smaller; the teeth are rather hypsodont and the incisor is not grooved. According to WOOD the species takes an intermediate evolutionary place between the American genera *Monosaulax* and *Amblycastor* (although, it is much smaller than the latter; it is on the contrary more

hypsodont. A.S.), "tending to associate these genera more closely than STIRTON (1935) considered probable." Because the affinity to *Monosaulax* is thought to be greater than to *Amblycastor* the Montana species has been termed *Monosaulax senrudi*, new species.

If of *Amblycastor* no more was made known than the dentition only, there was nothing to object against STIRTON's phylogenetic scheme (chart 2) pointing to the possibility of the descentance of *Trogotherium* from *Amblycastor* or from *Monosaulax eseri*. But the small size of the oldest species of *Trogotherium* known, much smaller than that of *Amblycastor*, together with its much less flattened limb bones, excludes *Trogotherium* from being a descendant of *Amblycastor*. Here, as in so many questions of phylogeny, the increase of our morphological knowledge is a spoilsport. For it must be excluded that so large a genus as the Upper-Miocene *Amblycastor* gave rise to a rather small form as the Upper-Pliocene *Trogotherium minus*, because in all lines of the Castoridae the size increases in the course of the Tertiary. And as regards "*Monosaulax*" (= *Steneofiber*) I stand by my opinion of 20 years ago that the presence of an articulation between fibula and calcaneum in *Trogotherium* excludes the descentance of the latter from *Steneofiber* (*Chalicomys*), because this articulation is already lacking in the Upper-Oligocene species *Steneofiber viciacensis* Gervais, like in all other members of the *Steneofiber-Castor* line.

It is again our ignorance concerning the form of the limb bones which makes us accept with some reserve the utterance of HEINRICH FREUDENBERG (a young promising scientist unfortunately fallen in the war): "Der Castoridenstamm wurzelt unzweifelhaft in den Theridomyidae; der älteste Castoride, *Chalicomys insignis*, aus den Phosphoriten des Quercy, ist selbst noch als Theridomyide zu betrachten." (1941, p. 157.) His material of *Theridomys* was rich indeed, existing in hundreds of lower and upper jaws with teeth, and his investigation thorough. However, he does not consider any other part of the skeleton, and referring to SCHLOSSER's opinion of 1902 p. 118, that *Steneofiber* "der Stammvater der Castoriden" must be derived from *Theridomys aquatilis*, FREUDENBERG disregards the fact that in a note at the end of the same publication (p. 144) SCHLOSSER renounces his opinion. "Es besteht zwischen *Theridomys* und *Steneofiber*, also den Castoriden, wirklich keine nähere Verwandtschaft, weshalb die gegentheilige Angabe – p. 118 – hinfällig wird."

When more than 20 years ago I discussed the phylogenetic place of *Trogontherium* with Professor MAX WEBER, showing this Master in comparative anatomy almost the whole skeleton of the Tegelen species; he neither had a solution for the problem, and considered it to be rather loosely connected to the true Beavers. So on p. 276 of the second edition (1928) of his standard work, *die Säugetiere*, we read: "In Europa lebte bis ins Plistocän das einer eigenen Familie angehörige, grosse *Trogontherium* Fisch."

Note on *Anchitheriomys*

1885. *Hystrix Wiedemanni*, ROGER, p. 109, Pl. 1, fig. 12; Reischenau, fragment of i inf.
1898. *Anchitheriomys Wiedemanni*, ROGER, p. 7, Pl. 3, figs 9, 10; Stätzling, fragm. of i. sup., calcaneum.
1902. —, SCHLOSSER, p. 24; Abbach, p₄ and fragm. of i inf.
1904. —, ROGER, p. 3, Pl. 2, figs. 6, 7, 10; Häder, fragm. of i, p⁴, m²—m³.*
1938. *Anchitheriomys wiedemanni*, SEEMANN, p. 1-55, 1 Pl., 35 textfs.; Viehausen, fragsm. of skull, mand. with p₄—m₂, max. with p⁴—m³, isolated teeth, fragm. of hum., ulna, rad., cerv. vert.
1902. *Steneofiber* spec., MATTHEW, p. 305, fig. 12; Pawnee Creek, Colorado, p⁴.
1902. ?Castorid indet., MATTHEW, p. 305, fig. 13; *ibid.*, p₄.
1909. *Hystriops* cf. *venustus*, MATTHEW and COOK, p. 381, fig. 10; Snake Creek, Nebraska, max. fragm. with p⁴ and m¹.
1918. *Amblycastor fluminis*, MATTHEW, p. 197, figs. 7, 8; Snake Creek, Nebraska, mand. with p₄, p₄.
1935. —, STIRTON, p. 411, figs. 47-52; Snake Creek, same specimens and mand. with i, p₄ (partly), m₁, some isolated lower teeth.

*) SEEMANN did not mention these teeth, although they perfectly agree with those figured on her fig. 32, but the latter dentition is younger than the molars from Häder; however, the size is the same, considering that ROGER's figures are natural size and those of SEEMANN 2.3 × nat. size.

Another tooth from Häder, not mentioned by SEEMANN, is an upper molar of a rodent, which was first referred by SCHLOSSER (1884, p. 28) to *Hystrix suevica* and later (1902, p. 24), although with doubt, to *Anchitheriomys*. According to the author the tooth is 7 mm long and 6.5 mm broad at the crown surface, and coated with a thick layer of cement. The figure (Pl. 3, fig. 27) shows a rather hypsodont molar, and this, together with its comparatively small size and the presence of cement, renders it highly improbable that this molar from Häder belongs to *Anchitheriomys*.

1934. *Amblycastor tungurensis*, STIRTON, p. 413, figs. 53-59; Mongolia, fragms. of skull, 2 fem., tib., calc., ulna, mand. with p_4 — m_3 , isolated teeth.

1939. *Amblycastor caucasicus*, ARGYROPULO, p. 636, 5 figs.; Savropol, Ciscaucasia, p_4 .

When reading ARGYROPULO's record (1939) of a lower premolar of a new large beaver (*Amblycastor caucasicus* sp. n.) from the Pliocene (more precise data wanting) of Southern Russia it is not well to understand why the author referred this tooth to *Amblycastor* and not to *Anchitheriomys*, the large Upper Miocene beaver, with brachydont teeth, described by ROGER (1885, '98 and 1904) and by SEEMANN (1938) from Bavaria which is so much nearer to Ciscaucasia than are America and Mongolia from where the Upper Miocene *Amblycastor* had been recorded.

The Russian premolar is a rather young tooth; the only premolar of *Anchitheriomys*, figured by SEEMANN (fig. 31) is older, having been worn off so far that the first and the last of the three inner folds have become isolated from the circumference of the tooth and form some islets on the crown surface. Judging from the figures the structure of both premolars is the same. Both consist of a rather narrow anterior and a broader posterior half which has a width of 11 mm in the German, and of 11.5 mm in the Russian, tooth. It can be seen on SEEMANN's fig. 33 that in all teeth the outer infold (STIRTON's hypostriid) approaches closely the root bases, as is described by ARGYROPULO regarding the Russian premolar, but it does not reach it. In the German tooth the middlemost inner infold appears to be somewhat longer open to the circumference of the tooth than are the anterior and the posterior folds, as is the rule in Castorids. On the inner side of the Russian premolar the three grooves appear to be of equal length. However, also in *Amblycastor fluminis* (MATTHEW, 1902, p. 305, fig. 13) the middle inner infold is longer open to the side than the posterior fold, and penetrates deeper into the tooth than does the anterior one.

Comparing in this way the Russian tooth with the literature on both, *Anchitheriomys* and *Amblycastor*, my presumption gathered strength, that these names represent species of one and the same genus. But then the generic name must be *Anchitheriomys*, as before and in 1904 ROGER had already made known of his

Anchitheriomys Wiedemanni some fragments of lower and upper incisors with the characteristic longitudinal grooves, three upper teeth and a calcaneum, whereas it was not until 1918 that MATTHEW established the name of *Amblycastor*. (This name was not chosen fortunately, as the supposed bluntness of the snout was based on the erroneous supposition that the length of the diastema had been no more than 9 mm. In reality this measurement is about 25 mm (STIRTON, 1935, fig. 50), which corresponds with a normal beaver snout.)

			<i>Amblycastor</i>					<i>Anchitheriomys wiedemanni</i>	<i>Trogontherium</i>		<i>Castor fiber</i>
			<i>fluminis</i>		<i>tungurensis</i>		<i>caucasicus</i>		<i>cuvieri</i>	<i>boisvilletti</i>	
STIRTON 1935			MATTHEW 1918	STIRTON 1934	ARGYROPULO 1939			SEEMANN 1938	REICHENAU 1910	SCHREUDER 1929	RUGER 1928
22393			f.7 type	f.8	f.1	f.2D			Mosbach aver.	Tegelen max.	Mosbach aver.
p ₁	1. length	15.5	15.8	14.0	12.5	12.0	14.8	12.5	13.3	14.0	10.0
	2. width	12.4	12.4	11.7	10.8	12.5	11.5	11.0	10.5	10.0	8.8
	1 : 2	1.25	1.27	1.19	1.16	0.96	1.29	1.15	1.16	1.4	1.14

The sizes of the teeth agree very well in the two Upper Miocene Castorids in question (see Table). The premolar of the Russian species appears to be rather long. That is why ARGYROPULO presumes that beaver to be two and a half times as large as *Castor fiber*; as regards the only tooth known this is disavowed by the Table above.

STIRTON's generic diagnosis of *Amblycastor* runs: Incisors with convex anterior face which is grooved longitudinally as in *Trogontherium* and in *Castoroides*; lower molars with a hypostriid and a mesostriid, neither extending to base of tooth; cheek teeth relatively low crowned; diastema of lower jaw normal, not short; large crescentic parafoasettid on anterior half of P₄; P₄ not as elongated as in other castorids.

This diagnosis fits exactly *Anchitheriomys* which, like *Amblycastor*, is an Upper Miocene castorid of about beaver size.

As a matter of cause the crescentic form of the parafoasettid is typical to a certain stage of wear only. On SEEMANN's fig. 31 p₄ has not been worn off far enough to show a crescent, but m₁ does show it indeed.

P₄ of *Amblycastor fluminis* appears to be not less elongated as that of other large castorids (see Table) as is shown by the measurements given by STIRTON (1935, p. 415), and the same holds good as regards the Russian species.

It is regrettable that of the limb bones there are but fragments (of *Amblycastor tungurensis*) to be compared with those of *Anchitheriomys wiedemanni*. My thanks are due to Professor G. G. SIMPSON, New-York, for some fine photographs of the former; as regards the latter they cannot yet reach us, so we must rely on the somewhat superficial description of SEEMANN. She writes that neither the mandible nor the humerus, nor the fragments of ulna and radius, show fundamental differences with *Castor*. The skull bones, just as in *Amblycastor*, are somewhat larger than in *Castor*. According to STIRTON the remains of *Amblycastor* indicate that it is a beaver with a larger head, but shorter limbs than *Castor*.

All in all the insignificant differences existing between *Anchitheriomys* Roger and *Amblycastor* Matthew are of specific, and by no means of generic, grade. My conclusion is that it is one and the same genus, *Anchitheriomys*, which lived throughout the northern hemisphere. STROMER (1940, p. 13) points to the fact that the genus *Meterix* (HALL, 1929) or *Plesiosorex* (VIRET, 1940), an erinaceid occurring in the Upper Miocene of Bavaria, as well as in that of Nevada, has not been found in East-Asia. The large castorid in question, on the other hand, has been found also in Mongolia, which links the occurrence in Colorado and Nebraska with that in Europe.

Summary

Three species of *Trogotherium* have existed in Eurazia. The geologically oldest is the beaverratt-sized Upper-Pliocene *Trog. minus* Newton of the lowest strata (Nodule Bed) of the Red Crag in East-Anglia and in the Astian fauna of Roussillon. It also occurred in Siberia. The type skull of the beaver-sized *Trog. boisvilletti* (Laugel) has been found in St. Prest, near Paris, and all the isolated skeleton bones are known from the *Trogotherium* Clay of Tegelen in the Netherlands, where this gnawer was abundant among an Upper-Villafranchian fauna (*Archidiskodon meridionalis*, (archaic type), *Eucladoceros*, *Sus strozzii*, *Leptobos*, *Miomys pliocaenicus*, etc.) of the Günz-interstadium. Of the third

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Trogontherium boisvilletti (Laugel), Tegelen, Concretion with hinder portion of skull. Nat. size. Coll. Teylers Museum, Haarlem, 15795.

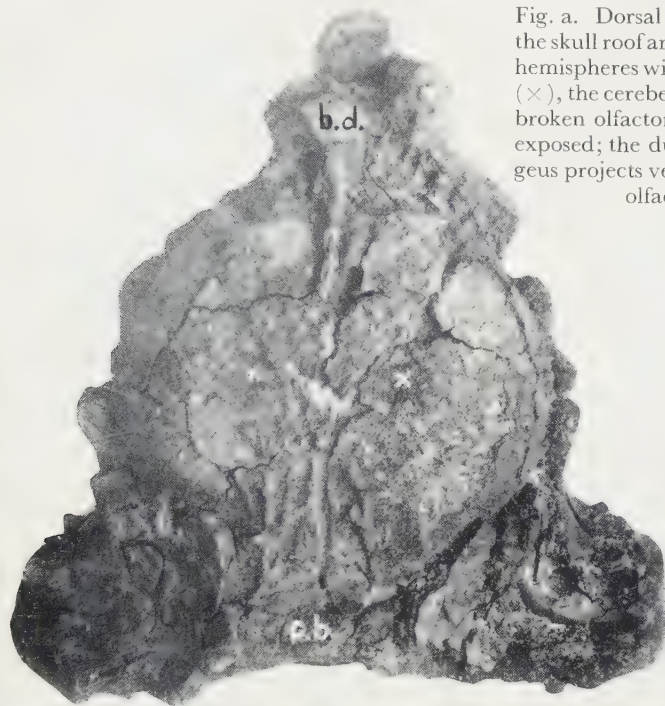


Fig. a. Dorsal view. The bones of the skull roof are lacking and so the hemispheres with the shallow dints ($\times$), the cerebellum (*c.b.*) and the broken olfactory bulbs (*b.ol.*) are exposed; the ductus naso-pharyngeus projects ventrally before the olfactory bulbs.

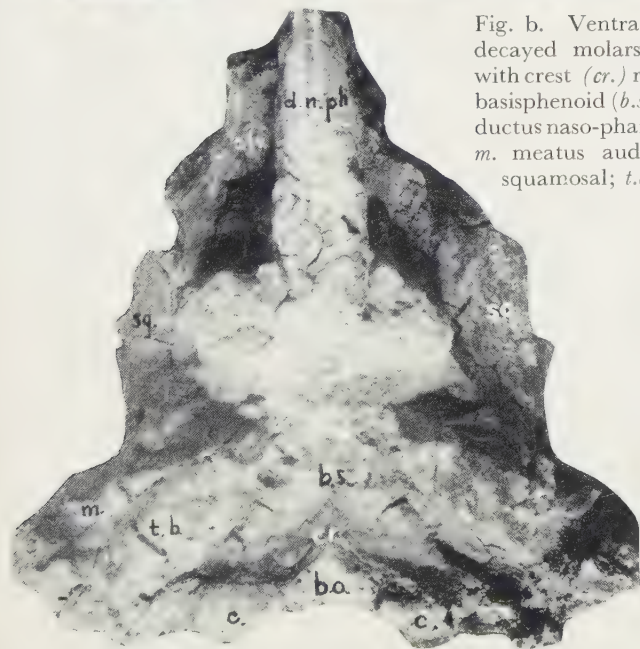


Fig. b. Ventral view. *alv.* place of decayed molars; *b.o.* basi-occipital with crest (*cr.*) running forwards to basisphenoid (*b.s.*); *c.* condyle; *d.n.ph.* ductus naso-pharyngeus (broken off); *m.* meatus auditivus externus; *sq.* squamosal; *t.b.* tympanic bulla.

Trogontherium boisvilletti (Laugel), Tegelen. Concretion with skull; $\times 3/4$.
Coll. Teylers Museum, Haarlem, 15794.

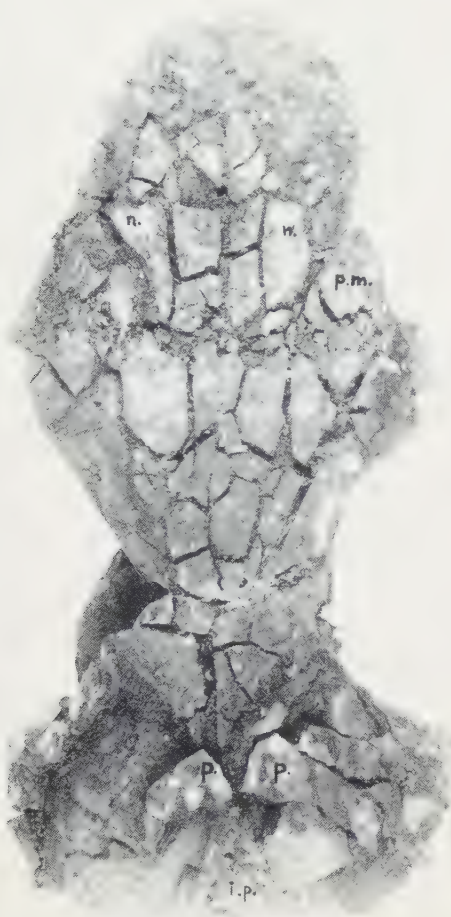


Fig. a. Dorsal view. *n.* nasal bones;
p.m. premaxillary; *p.* parietal; *i.p.*
interparietal.



Fig. b. Ventral view. *b.a.* base of
alveole of the left incisor; *i.* Left
incisor; *a.z.a.* anterior end of the
broken zygomatic arch; *a.z.p.* posterior
end of the latter; *pm* right
premolar.

Trogontherium boisvilletti (Laugel), Tegelen. Concretion with cast of skull.
 ± $\frac{1}{2}$. Coll. Rijksmuseum v. Miner. en Geologie, Leiden, St. 47497.

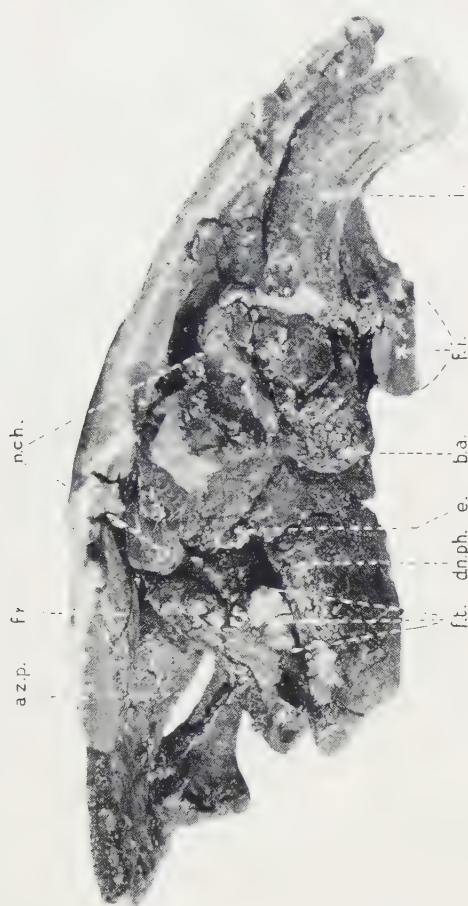


Fig. a. Side view, after removal of the right half of the crust and of the endocranial cast.



Fig. b. Ventral view, idem. *f.i.* cast of the foramina incisivi; *b.a.* bottom of the alveole of the incisor; *d.i.* ductus incisivus; *e.* place of the decayed ethmoid; *fr.* impression of the frontal bone on the innerside of the crust; *n.ch.* cast of nasal chamber; *f.t.* cast of the canalis transversus in the praesphenoid; *a.z.p.* posterior end of the decayed left zygomatic arch.

Trogontherium boisvilletti (Laugel), Tegelen. Endocranial cast out of the concretion in the Leiden Museum, St. 47997, $\times 3/4$.

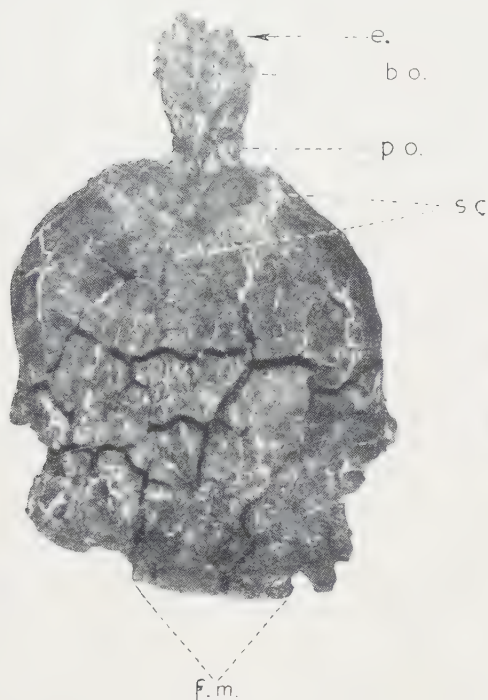


Fig. a. Dorsal view. *e.* place of the lamina cribrosa through which the fila olfactoria (broken off) pass from the olfactory bulbs (*b.o.*) to the nasal chamber; *s.c.* impression of the somewhat dislocated sutura coronalis; *f.m.* foramen magnum.



Fig. b. Ventral view. *V* nervus trigeminis; *VIII* nervus acusticus; *f.l.a.* cast of foramen lacerum anterius

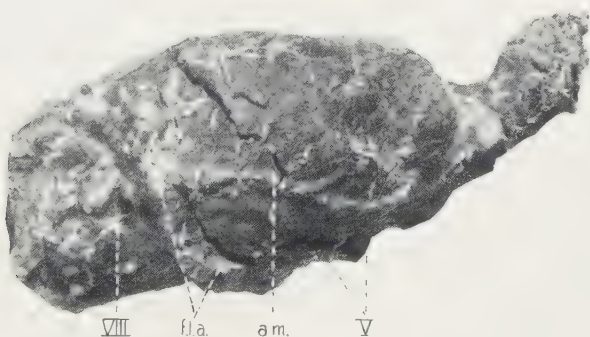


Fig. c. View of right side. *a.m.* arteria meningea.



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COMPTES RENDUS DE LA SOCIÉTÉ NÉERLANDAISE DE ZOOLOGIE

AOÛT 1943—JUILLET 1945

Séance du 30 octobre 1943 à Leyde

1. H. TRAMPUSCH (Amsterdam). Contribution to the teratogenesis of the head.

The significance of teratologica for a right understanding of morphogenesis is here shown with the help of an anencephalous human foetus. To use this to its full extent a discription alone is not sufficient; the importance of a causal analysis should not be underrated. This purpose is served best by experiment. After discussion of the literature important for the experimental teratogenesis of the head, stressing especially such disturbances of development which are the result of chemical (LiCl, cyanide etc.) or physical (radium ultraviolet, and X-rays) means, experiments are described which, with improved methods, lead to results that bring to light the causal relations of the deformity.

These experiments consist of irradiation of blastulae of axolotls with different doses of X-rays, showing that by the use of exact doses, — granting the reproducibility of the experiments — the defects are in proportion to the amount of the rays used. The animals developed from the radiated eggs show all the intermediate stages from slight microcephaly to complete acephaly, as well as deformities of the organs of special sense and of the gill-region on a larger or smaller scale. ¹⁾

The histological examination of the germs so treated, together with the deformity of the region of the head and the gills, shows a well-marked retardation in the differentiation of parts of the chorda. This is recognized by the greater quantity of yolk grains in such cells. Furthermore, the prechordal material is abnormal in its distribution on the base of the skull and more or less clumped, while its histological differentiation is also extremely defective.

Besides these morphological disturbances we notice in the series of sections of the germs under experiment scattered pyknotic

¹⁾ A detailed account of these experiments will be found in an article in *Acta Neerlandica Morphologiae*, vol. 6, 1946.

nuclei the density of which varies at different levels of the specimen, as well as in different animals. To examine this behaviour a new series of axolotl blastulae was irradiated with 300 r, a dosis which earlier experiments show to be sublethal. The animals thus treated were preserved at different intervals from the time of irradiation. The number of pyknoses per section were counted after staining with borax carmin which brings out the pyknotic nuclei, and their density with respect to the unit volume was measured, after the circumference of the section had been found with the help of a planimeter.

If these numbers are plotted in a coordinate system the peaks in every case are in the prechordal region of the skull, irrespective of the dose of irradiation. It can be noticed that the decline of the curve is more or less steep craniad and caudad, in accordance with the dose and the stage of development in which the animal is found. It follows from this distribution of pyknoses that the prechordal part of the skull suffers most from the irradiation, while neither the brain nor the eyes, morphological deformity of which can go very far, contain many pyknotic nuclei.

According to the findings of VOGT (1929) and his fellow-workers in their kinematic examinations it is known that the material of the prechordal plate in the initial stage of gastrulation — the stadium in which our experiments have taken place — is about to begin its invagination round the edge of the blastoporus. Therefore one can suppose that its morphological activity is accompanied by a local increase of metabolism. The experiments of BERGONIE and TRIBONDEAU (1906) show that a greater susceptibility to the effects of radiation follow this increased burning up process. This leads to the hypothesis according to which general irradiation with sublethal doses causes injury to the praesumptive prechordal material. On the other hand again, as a consequence of this injury the prechordal material is unable to fulfill its morphogenetic function, i.e. the organisation of the presumptive material of the head, as is shown by ADELMANN (1933), MANGOLD (19 . .), and others.

In view of the above-mentioned experiments the causal connection between the disturbance in the development of the germs and the check, or injury by rays of the prechordal plates exists without doubt. Microcephaly is, therefore, not to be taken as an expression of direct injury through rays, but as a deficiency in organisation which, for its part, is caused by injury through rays.

In the same way the defects or deformities of eyes, ears, gills, respectively, are explained as consequences of defective organisation (impaired formation of neural plates, deficiency in the neural crest, etc.), and caused by the rays administered in an indirect manner only.

This explanation of teratogenesis of the head is strengthened by further observation, which shows that more intensive irradiation of amphibian blastulae causes more or less far-reaching disturbances in invagination of the germ during gastrulation, which, in extreme cases, may lead to total exogastrulation. Here, too, the extent of the suppression of normal movement during development is in proportion to the intensity of the radiating energy.

2. L. D. BRONGERSMA (Leyde): Demonstration of the Oudemans bequest.

Dr. A. C. OUDEMANS, who died on January 14th, 1943, bequeathed to the Netherlands Zoological Society his archives relating to the Great Sea Serpent. Although it cannot be said that professional zoologists accept the existence of the Great Sea Serpent as an indomitable fact, OUDEMANS at least was a firm believer in it. In 1889 he started to study all available evidence. This resulted in his publishing privately a voluminous book, entitled: *The Great Sea Serpent, an historical and critical treatise* (Leiden & London, 1892, XV + 592 pp). In this book all accounts that came to his notice are discussed; the exterior characters and habits are described, and finally the systematic position of the animal is dealt with. His conclusion is that the Great Sea Serpent is a very large Pinnipede, for which OUDEMANS uses the name *Megophias megophias* (Raf.). In the years to follow OUDEMANS kept on collecting any information he could get on this subject. This resulted in a large collection of some thousands of press cuttings, letters, drawings and reprints from scientific journals. On the reported appearance of the Loch Ness animal, OUDEMANS collected every scrap of information about this too; on this subject alone his archives contain 1024 items.

Whatever the opinion among zoologists may be, whether they consider *Megophias megophias* as existing only in the brains of phantastic sailors, or as a reality, there is no doubt that the OUDEMANS bequest presents us with an extremely interesting collection of information on this subject.

A number of the most interesting drawings, letters and press cuttings were demonstrated.

3. J. J. TESCH (Leyde). On the horizontal distribution of Thecosomata (shelled Pteropods) in the Atlantic and its bearing on bipolarity.

Data on distribution were obtained by an analysis of samples of the "Dana"-expeditions 1921-22 and 1930. Simultaneous fishing with a series of pelagic nets at different horizontal levels, mostly during two hours, enabled the lecturer to calculate the average number of individuals caught per fishing unit, and it was possible to demonstrate that some species, while not entirely avoiding the waters between 10 N. and 10 S. lat., nevertheless show a tendency to crowd towards higher latitudes; the next phase being a severing of a hitherto connected habitat and a discontinued spreading of wholly or nearly wholly identical species inhabiting subarctic and subantarctic regions. In a few cases this process leads to truly bipolar species. This would explain why bipolar species, originating from typical warm water ancestors, are isolated examples of a gradual expansion towards polar waters; it would also explain why subantarctic waters, with their broad front towards southern tropics, contain more endemic forms than boreal regions, where ecological conditions are different. Truly abyssal species of Thecosomata are cosmopolitic and cannot be used to elucidate the bipolarity phenomenon.

Les considérations données ci-dessus (avec des notes sur l'anatomie etc.) sont empruntées d'un ouvrage, intitulée "The Thecosomata. I. The Atlantic", qui sera incluse dans la série "The Carlsberg, Foundation's Oceanographical Expeditions around the World 1928-30 and previous "Dana-expeditions, Copenhagen".

Séance du 11 décembre 1943 à Utrecht

1. A. D. VOÛTE (Hoenderloo). La régulation des populations des insectes dans les forêts vierges et les autres forêts.

Voir: Archives Néerlandaises de Zoologie, Tome VII, p. 435.

2. N. POSTMA (Utrecht): L'influence du ganglion sus-oesophageal sur les fonctions du pied d'*Helix pomatia* Linné.

En opposition aux résultats obtenus précédemment l'orateur

a constaté que le ganglion cérébral a une influence secondaire sur la réaction tonique du pied de l'escargot. Selon l'activité du ganglion ce centre diminue l'activité des centres tonolytiques et excitateurs, se trouvant dans les ganglions pédaux de l'escargot, ou le ganglion céphalique active le centre inhibiteur du tonus musculaire et le centre renforçant le tonus. Voir pour les détails Arch. Néerl. de Zool., tome VII, p. 471.

Séance du 22 janvier 1944 à Amsterdam

E. H. HAZELHOFF (Groningue). Investigations concerning the comparative physiology of respiration.

A. In many Teleosteans as much as 60-80% of the oxygen contained in the inspired water is withdrawn from it by one passage along the gills (oxygen utilization 60-80%). Three factors co-operate to render the gill-apparatus so highly efficient:

1. The gills form a sieve without a single large opening, so that each water particle is compelled to flow through one of the extremely narrow "meshes" between the very numerous gill lamellae;

2. The blood current in and the water current between the gill lamellae flow along each other according to the counter-current principle; and

3. The water current flows continuously, not intermittently, along the gill lamellae.

A high utilization is also found in most other gill-breathing animals. A low utilization (about 5-15%) is only found in Lamellibranchs, Ascidians and other particle-feeding animals. The explanation is that in these groups a very strong water current along the gills is required to assure the capture of a sufficient amount of food.

B. The regulation of breathing is effected in at least five different ways:

1. In all gill-breathing animals and in many animals breathing by lungs or tracheae: regulation of the respiratory movements (regulation of ventilation).

2. In nearly all insects: regulation of diffusion in the tracheae by opening or closing the tracheal valves.

3. In all or at least in very many insects: regulation of diffusion by the extension and withdrawal of the air columns in the tracheoles.

4. In some species, e.g. in many fresh-water fishes, *Eriocheir sinensis*, etc.: emergency breathing.

5. In bees and other social Hymenoptera: social breathing regulation by fanning at the entrance of the hive or nest.

C. In the bird's lung a continuous air-current is flowing through the parabronchi both during inspiration and expiration; in both phases the direction of the current is the same (viz., from the dorsobronchi through the parabronchi to the ventrobronchi). The valve(s) or sphincter(s) formerly supposed to be present in each lung do not exist; the air currents are guided only by the aerodynamical effects caused by the very peculiar anatomical structure of the lung (i.a. by the arrangement of the laterobronchi just opposite the entrance to the dorsobronchi).

D. In the seal, beaver, penguin and duck the heart rhythm is slowed down considerably during shallow diving; the oxygen contained in the blood is used up by the central nervous system (brain and *medulla spinalis*), the muscles and probably also the abdominal organs receiving little or no blood (SCHOLANDER, 1940). — In a Cetacean diving to a moderate depth of say 20 or 30 meters the blood vessels outside the thorax, the spinal canal and the *cranium* are exposed to a greater pressure than those inside these spaces. At this depth the *arteria carotis interna* and the *vena subclavia interna* are probably compressed, but the brain can still be supplied with blood by special vessels, well-developed in all Cetaceans, running to and from the brain by way of the spinal canal and the *foramen magnum*.

Balaenoptera musculus, *B. physalus*, *Hyperoodon ampullatus* and *Physeter macrocephalus* sometimes dive to a depth of 100 meters or even considerably more. In these species the *ductus Botalli* remains open throughout life. It is assumed that the circulation through the brain and *medulla spinalis* is now kept up by the right ventricle, the blood flowing from the right ventricle through the *arteria pulmonalis*, *ductus Botalli*, *aorta*, *arteria costocervicalis*, *rete arteriosum thoracicum*, *arteriae meningeae spinales*, brain capillaries, *sinus basilaris*, *rete venosum occipitale dorsale et ventrale*, *venae columnae vertebralis*, *vena costocervicalis*, *vena cava anterior* and right atrium back to the right ventricle. The closure of the *foramen ovale* does not impede this type of circulation.

Séance du 19 février 1944 à Utrecht

1. J. W. SLUITER (en collaboration avec G. J. VAN OORDT) (Utrecht). Recherches expérimentales sur la fonction de l'interstitium de la glande sexuelle.

Voir: Experimentelle Untersuchungen über die Funktion des Interstitiums der Gonade. I. Versuche an männlichen und weiblichen juvenilen Mäusen. Zeitschr. Zellforschung, vol. 33, pp. 311-335, 1945.

2. M. F. MÖRZER BRUYN (Utrecht). Die Gastropodenfauna einiger Niederländischen Wälder.

Die vorliegende Abhandlung ist ein Teilstück einer grösseren biosoziologischen Arbeit, die die Erforschung der niederländischen Biocoenosen zum Ziel hat.

Eine Biocoenose ist, kurz gefasst, eine Lebensgemeinschaft von Pflanzen und Tieren, die in einem einheitlichen Abschnitt der Biosphäre, einem oekologisch homogenen Gebiet, dem Biotop, lebt.

Einerseits wurden sogenannte Kulturwälder, also Kiefern-, Fichten-, Lärchen und Buchenwälder untersucht, anderseits die natürlichen Wälder, den Pflanzensoziologen bekannt als Querceto roboris-Betuletum, Querceto-Carpinetum, Alnetum, Betuletum pubescentis u.s.w.

Die forstwirtschaftlich wichtigen Kulturwälder sind fast alle auf nahrungsarmen sauren Sandboden gepflanzt worden. Wir können sie deshalb an besten mit dem Querceto roboris-Betuletum-Wald vergleichen.

Die Gastropodenzahl wurde quantitativ bestimmt. In jedem Waldtypus wurde die Anzahl lebender Tiere pro m² festgestellt.

Die eutrophen und feuchten Wälder haben eine weit grössere Schneckenfauna, als die Wälder auf oligotrophen und trockenen Boden. Es konnte festgestellt werden, dass viele Schneckenarten, ihrer oekologischen Valenz gemäss, wenigstens lokal als Charakterarten angesehen werden dürfen.

Im oligotrophen nassen Betuleto-Salicetum wurden nur 3 Arten gefunden, im mesotrophen, nassen Betuletum pubescentis 11, im eutrophen Alnetum 26. Im oligotrophen feuchten Querceto roboris-Betuletum molinietosum 5 Arten, im feuchten eutrophen Querceto-Carpinetum stachyetosum 35. Im trockenen oligotrophen Querceto roboris-Betuletum 15 Arten; eutrophe trockene Wälder wurden nicht untersucht.

Die Kulturwälder sind fast ohne Ausnahme sehr Schnecken-arm; fast immer wurden nur 2-3 Arten gefunden. Wahrscheinlich ist der saure Humus der Kiefern-, Fichten- und Buchenwälder schuld daran.

Später werde ich über das Vorkommen und die Verbreitung der Schnecken in diesen verschiedenen Waldtypen noch ausführlicher berichten.

3. E. J. SLIJPER (Utrecht). L'anticlinie des apophyses épineuses chez les mammifères.

Voir: Comparative biologic-anatomical investigations on the vertebral column and spinal musculature of Mammals.

Verh. Kon. Ak. Wetensch., 2e sectie, vol. 42, no. 5, 1946.

Séance du 1 avril 1944 à Amsterdam

1. J. KLOOS (Utrecht). Induction par les parties médiales et latérales du toit de l'archentéron.

Voir: Acta Neerlandica Morphologiae normalis et pathologicae, vol. 5, p.

2. C. ROMIJN (Utrecht). Foetal respiration in birds.

The volume of the air space of the hen's egg increases in a rectilinear relation to the time of incubation. At the end of this period a volume of 11 cc. may be reached, whilst in the fresh fertile egg it does not exceed some tenths of a cc.

A method has been described by which the composition of the gas found in the air space could be determined in one and the same egg throughout the time of incubation. The foetus remains under fully physiological conditions during the experiments. The pressure in the air space was measured in one and the same egg continuously during the time of incubation and was found nearly atmospheric.

When the chick leaves the egg, it has terminated its foetal state about 24 hours previously. During these hours, the parafoetal period, respiration in the air space by pulmonary breathing has been added to allantoic respiration. The respiratory movements of the animal during this period could be recorded optically. An exceedingly high carbon dioxide content is present in the air space (up to 9.11%) during the parafoetal period and a very low oxygen percentage (9% or less).

The permeability for different gases of the shell with the membranes has been measured with an accurate differential method.

During the incubation time the permeability of the shells of fertile eggs increases; the part of the shell which covers the air chamber becomes more permeable for gases than the other part. The wet shell membranes are limiting factor in the oxygen supply of the foetus; increasing permeability is at least for the greatest part result of exsiccation of the membranes. The oxygen consumption of the foetus could be calculated from the diffusion data obtained and the result was in good accordance with the figures found in literature.

Les recherches sur la chambre à air ont été publiées dans le *Journal of Physiology*, vol. 94, p. 365 (1938), dans quel journal je publierai aussi les résultats des autres recherches.

Séance du 13 mai 1944 à Leyde

1. P. KORRINGA (Bergen-op-Zoom). Structure et croissance de la coquille de l'huître.

La structure de la coquille de l'huître diffère considérablement de celle du type classique Anodonta. Le périostracum de l'huître, qui très souvent échappe à l'attention, est extrêmement mince et transparent; il est en outre très élastique et extensible, de sorte que la bordure du manteau peut se retirer facilement de celle de la coquille. Ce n'est que la valve supérieure de l'huître qui possède une couche prismatique sous forme d'écailles brunes et élastiques, disposées de façon imbriquée, qui jouent un rôle dans la fermeture hermétique de la coquille. Malgré leur élasticité on a constaté que les écailles ne se composent que de 4% de matière organique (conchyoline) et de plus de 95% de calcite. Les prismes sont peu réguliers et disposés obliquement. La masse proprement dite de la coquille est formée par la couche lamelleuse subnacrée. Celle-ci se compose de 1% de conchyoline et pour le reste presque entièrement de calcite. Cette couche lamelleuse subnacrée se compose en majeure partie d'une masse blanche assez dure et d'un éclat terne, qui s'écaille facilement, et qui, examinée de plus près, paraît consister en agrégations écailleuses de feuillets de calcite très fins. En outre on trouve dans la coquille des amas dits "crayeux" assez tendres d'un blanc mat, qui à l'examen se présentent en petits feuillets accolés disposés verticalement. C'est de ces amas, dits "crayeux", et qui contiennent un certain pourcentage d'eau de mer, que l'huître se sert comme remplissage avec lequel elle façonne la forme intérieure de la coquille selon

ses besoins, indépendamment de la forme extérieure de la coquille.

Nos lumières sont encore assez faibles sur la manière, dont les tissus vivants sécrètent la coquille. Il est même impossible d'indiquer avec certitude les cellules qui fournissent la sécrétion calcaire. Il est probable que les cellules glandulaires que j'ai découvertes dans la bordure du manteau de l'huître y jouent un rôle. Les théories biochimiques sur la sécrétion calcaire sont encore très peu satisfaisantes.

Nous sommes mieux instruits sur la périodicité de la croissance de la coquille. Ainsi j'ai constaté que les huîtres Zélandaises commencent à épaissir leur coquille aussitôt que l'eau atteint 10°C , que la "pousse" est formée à des températures au dessus de 15°C et que le poids de la coquille continue à augmenter tant que la température de l'eau reste au dessus de 10°C .

2. D. A. HOOIJER (Leyde). On the rhinoceroses and the tapir from prehistoric caves in Sumatra, and on their supposed occurrence in Borneo.

Material from prehistoric caves in the Padang Highlands, Sumatra, was collected by EUG. DUBOIS in the years 1888 to 1890, but for the greater part it remains still undescribed. It consists mainly of loose teeth, the roots of which have been gnawed off by porcupines; many of them are in a fragmentary condition.

The two recent rhino's from Sumatra, viz., *Rhinoceros sondaicus* Desmarest and *Dicerorhinus sumatrensis* (Fischer) are now shown to be represented in the cave-collection by teeth which often exceed their recent homologues in size. The latter species is four times as abundant as the former. Attention is drawn to the specific dental characters of the two forms, and it is shown that the upper molars from a cave deposit in Sarawak, Borneo, described by BUSK in Proc. Zool. Soc. for 1869 as *Rh. sondaicus* Desmarest in reality belong to *D. sumatrensis* (Fischer). The latter species, in contradistinction to the former, is known to occur in the recent state in Borneo as well.

As regards the presence of a tapir in Borneo, though noted by many authors, there is no direct evidence whatever. It can safely be struck off the list of the Bornean fauna. In the prehistoric tapir of Sumatra we observe an excess in size over recent *Tapirus indicus* Desmarest, but otherwise their characters are similar. In fact, the subfossil form almost completely fills the gap that exists between

the recent Malay tapir and *Tapirus augustus* Matthew et Granger from the lower Pleistocene of Wanh sien, China, and Tonkin, Indo-China. The latter species, however, is distinct from *T. indicus* Desmarest in its proportionally broader upper molars and in the more molariform anterior premolars.

3. A. PUNT (Utrecht). Gas-exchange in parasitic insects.

By means of a diaferometer the gas-exchange of several parasitic insects (bugs) was measured, in order to find out whether metabolism has changed markedly at that range of temperature which is called "the preferendum".

This could not be established.

On the other hand it turned out, that CO_2 -production in *Triatoma rubrofasciata* (and other insects) is rhythmical, the frequency increasing with rise of temperature (from about 3 periods per hour at 15°C. up to about 40 per hour at 36°C.).

Humidity of the air was of no importance.

In other insects too, even in pupas of Lepidoptera the same rhythmical CO_2 -production can be found.

More about this subject can be found in: A. PUNT: De gaswisseling van enkele bloedzuigende parasieten van warmbloedige dieren (Onderzoekingen van het Physiol. Lab. Utrecht, 8e R. III, '43).

COMPTES RENDUS DE LA SOCIÉTÉ NÉERLANDAISE DE ZOOLOGIE

OCTOBRE 1945—DÉCEMBRE 1948

Séance du 27 octobre 1945 à Leyde

1. J. J. TESCH (Leyde). Obituary, Dr. H. C. REDEKE 1873-1945.

On the 10th of April 1945 one of our most distinguished hydrobiologists died after a long illness, which during the prolonged stress and strain of the war years finally proved fatal. His prominent personality in his best years will remain in the mind of the numerous people with whom he came into contact, and many who were guided by him in the study of interesting and fascinating biological problems will keep a grateful remembrance of the man who up to the last days of his life, against all odds, stuck to his post as an active scientist. His last great study, a general introduction to the Hydrobiology of the Netherlands, could unfortunately not be terminated, but his literary legacy of 265 larger and smaller papers witness to his unimpaired scientific perseverance, even after the retirement from his official post in the beginning of 1933.

On the occasion of his reaching his 70th birthday this periodical published, by the hand of Dr. H. ENGEL, an appreciative account of the life and work of REDEKE, for many years Director of our Zoological Station, the founder of the Hydrobiological Club, Honorary Member of the Dutch Zoological Society, Delegate for the Netherlands in the International Council for the Exploration of the Sea, not to mention the many other social and scientific functions on which he was engaged and where his editorial and artistic talents stood him in good stead. The present writer, who in his youth was Dr. REDEKE's assistant, in the period when circumstances still allowed an active participation on our part in the programme of International Fishery Research, had frequent occasion to testify to the invaluable qualities of the leader whom we now commemorate, but he does not feel qualified to a judgment of REDEKE's later accomplishments. He is of opinion, however, that the account mentioned above will compensate for the gaps in his personal experience and that a renewed summing up of REDEKE's life work may be dispensed with.

In every life there are lights and shadows. Shadows, disappointments, did not fail in REDEKE's life. It is tragical that circumstances in the period between the two world wars prevented him from unfolding his wings quite freely, as he should have wished. Compelled, since 1924, to abandon the work for the International Fishery Research to others, he devoted himself to the study of limnological and brackish water life in our country and found a great consolation in this interesting field, where he may be called a pioneer and a reliable guide, and in which he retraced his first steps as a scientist, when, during his last student years, he investigated the plankton of the Zwanewater near Callantsoog, for which he was awarded a gold medal of the *Hollandsche Maatschappij der Wetenschappen* at Haarlem.

His was a forceful and yet kind and generous mind, erudite and cultured and well versed in literature, with a fine taste of music. He was aristocratic, in the best sense of the word, masterful at times, but always ready to see the other man's view in discussion. Convinced that his outer appearance should be in accordance with his inmost self he was always prim and neat, never allowing himself to be carelessly dressed. However busy, his working desk and his laboratory were a model of cleanliness. He had a keen sense of humour and was fond of "esprit". His loss will be keenly felt by all who knew him intimately.

2. H. J. VONK (Utrecht). In memoriam H. J. JORDAN, Professeur à l'Université d'Utrecht.

Voir: In memoriam H. J. JORDAN, Hoogleraar aan de Rijks-Universiteit te Utrecht. Utrecht, N.V. A. OOSTHOEK, 1946.

3. S. DIJKGRAAF (Groningen). Biographie de E. H. HAZELHOFF.

Voir: Jaarboek Kon. Nederl. Akad. v. Wetensch. 1944/45, p. 148-153.

Séance du 15 décembre 1945 à Utrecht

1. S. DIJKGRAAF (Groningen). How bats perceive their surroundings.

As SPALLANZANI showed in 1794 flying bats after being blinded avoid obstacles quite as well as they did before. According to a generally accepted theory the animals should possess an excessively developed tactile sense, enabling them to perceive and

locate approaching obstacles by means of certain air movements. In order to check this opinion experimentally the wings of a blinded bat were rendered insensible by cutting the brachial nerves. Yet obstacle avoidance proved to remain normal. On the other hand bats showed the ability of object perception at a distance not only when flying, but also when crawling about or without moving at all (sitting, hanging). In the latter case tactile stimulation can be excluded a priori, since the required air movements are lacking. Obstacle avoidance and object perception in general proved to be a function of the sense of hearing. Bats which were rendered completely deaf flew anxious and showed no longer obstacle avoidance. Every flight ended with a clumsy collision and even big objects like walls could not be detected by the animals.

Careful examination of bats in captivity showed them to make frequently characteristic "exploring" head movements, which always were accompanied by the production of a soft "rattling" sound, emitted from the bat's mouth and directed straight forward. Flying bats rattled incessantly. Only objects coming into this "sound beam" became detectable to the animals. Covering the mouth with a paper cap affected the ability of object perception in a striking manner. The frequency of the rattling sound (number of "clicks" per second) and its intensity increase every time a bat needs careful examination of an object, for instance when the animal is about to land. From these and other facts it may be concluded that bats perceive and locate the objects surrounding them acoustically by means of the reflected rattling sound. Their ability in object discrimination on this basis is studied at present.

As GRIFFIN and GALAMBOS showed recently, each click of the rattling sound consists of two components: an audible and a supersonic one. It is not yet clear to what extent each of these components has a functional meaning, though probably they will prove to be complementary to each other.

2. G. STIASNY (Leyde). Die Andesitlinie als Faunengrenze zwischen Indo-Westpacific und Ostpacific.

I. Einleitung.

1. Das Problem. Vorsprung der Geologen. Kein einheitliches Erklärungsprinzip bei den Biologen. Widerstreit

bei Geologen und Biologen und zwischen beiden Lagern. Gemeinsame Arbeit beider Teile nötig. Einseitigkeit der biologischen Forschung. Schwierigkeiten bei der Problemwahl. Die Andesit-Linie und nicht die "ostpazifische Sperre" Ekmans als Faunengrenze zwischen Indowestpazifisch und Ostpazifisch. Zusammenfassende Uebersichten im Schriftum erleichtern die Stellungnahme. Bisherige Einteilungen in Regionen des südwestpazifischen Gebietes und des ganzen Pacific.

II. Geologisch-Geographischer Teil.

2. Die Andesit-Linie in ihrer geologischen Bedeutung als der wahre Rand des Pacific. Ihr Verlauf.
Die Nephelin-Linie. Der Ostpazifisch. Der Indowestpazifisch. Oberflächen- und Tiefenströmungen. Die Sedimente.
3. Die "Ostpazifische Sperre" EKMANS. Kritik und Ablehnung derselben auf Grund geologischer und biologischer Argumente.

III. Biologischer Teil.

4. Hinweise in der biologischen Literatur auf die Andesit-Linie als Faunengrenze.
Die Andesit-Linie schon lange unter anderem Namen bekannt, aber in ihrer vollen Bedeutung als tiergeographische Grenze noch nicht erkannt.
5. Spiegelt sich die Andesit-Linie in der Verbreitung der marinen und terrestrischen Lebewelt des pazifischen Gebietes ab?

Beispiele aus dem Physical Atlas von BARTHOLOMEW und aus der "Tiergeographie des Meeres" von EKMANS, belegt durch viele Verbreitungskärtchen, Schwierigkeiten bei Samoa und Hawaii. Weitere Belege aus der übrigen Literatur bei verschiedenen Tiergruppen zu gunsten der Andesit-Linie als Verbreitungsgrenze.

6. Die Verbreitung des Genus *Anguilla*.
Der historische Standpunkt bei der Beurteilung der geographischen Verbreitung als nicht der alleinseligmachende kritisiert.

IV. 7. Kurze Zusammenfassung. Die Andesit-Linie als Hauptverbreitungsgrenze im pazifischen Gebiete charakterisiert.

Séance du 26 janvier 1946 à Amsterdam

1. P. D. NIEUWKOOP (Utrecht). Experiments concerning the regional determination of the central nervous system ¹⁾.

This investigation has started from the analysis of a transplantation series made to elucidate the origin of the germ cells in the Urodeles. It forms only a preliminary investigation, as this material had not been treated for study of the structure of the nervous system. The material consists of a thirty duplicitas larvae, reared for about 4 weeks after transplantation of the dorsal blastoporal lip in place of the ventral one at the early gastrula stage (experiment of SPEMANN '18). The primary embryos show a normal brain, whereas the secondary ones have very interesting nervous systems, varying between a complete nervous system and a spinal cord fragment only.

From this material I conclude that besides a mediolateral regulation (formation of a bilateral symmetric rudiment from an asymmetric graft) probably also a craniocaudal regulation (regulation of the missing caudal parts from more cranial ones) has taken place in the archenteron roof. Moreover, we may divide the analysis of the determination of the central nervous system into two fields of investigation, a) the determination of the ground plane of the central nervous system, formed by the "primary", always present, centers and commissures of each brain-part, b) that of the "secondary", more variable, specific formations in the brain (eye, Infundibulum, etc.). This analysis shows the important fact that the nervous system is determined in a large number of successive zones (at least 7 in the brain).

The results induced me to the formation of the following workinghypothesis: From the literature it is known that during gastrulation the power of regulation quickly decreases, whereas, simultaneously, the differentiation tendencies markedly increase in the archenteron roof. There are several indications that gradual, but fundamental, changes take place in the character of the determination process itself during gastrulation (cf the final conclusion). A critical consideration of the literature makes it probable that the spatial configuration of the neural plate is determined by an interaction between a strong regional "structure" of the archenteron roof and a weak regional "structure" of the ecto-

¹⁾ See also: Journ. of Exper. Biol. V. 24, p. 145-183, 1947.

derm. Both "structures" are probably based on the distribution of specific chemical substances. In my opinion, the open question, whether the regional influence of the archenteron roof reposes on a gradient of one chemical substance or on the presence of a number of specific inductors, has been nearly solved; there are many strong arguments showing that the initial induction phase is dominated by quantitative differences. According to me the spatial configuration of the nervous system may be explained by an equilibrium reaction between a strong mesodermal and a weak ectodermal gradient. Each brain-part is primarily determined by definite values of the reaction product.

These quantitative differences in the gradient must, however, finally lead to the formation of qualitatively different brain-parts. We may visualize this transition in analogy to the formation of closely related chemical substances with different proportions of the reacting components. In this way the equipotential neural field disintegrates into a number of vaguely limited sub-fields, which are more and more circumscribed and subdivided in following links of this chemical chain-process, leading to the ultimate spatial configuration of the nervous system. The determination process gradually changes from a purely quantitative (initial-phase) into a qualitative one (end-phase).

I will finally point to the significance of these incomplete nervous systems for the analysis of the causal relations between the brain-parts and between brain and sense-organs.

2. J. LEVER and L. M. VAN NIEUWENHOVEN S. J. (Utrecht). Experimental oviposition in *Limnaea stagnalis* L.

Voir: Monographs on the progress of research in Holland. M. W. WOERDEMAN and CHR. P. RAVEN, Experimental Embryologie, p. 72-74, Elsevier, 1946.

3. J. W. SLUITER and G. J. VAN OORDT (Utrecht). Experimental data on the function of the interstitium of the gonads: experiments with cockerels.

Voir: Quart. Journ. of Microsc. Science Vol. 88, p. 135-150, 1947.

Séance du 2 mars 1946 à Amsterdam

C. J. VAN DER KLAUW (Leyde). Ecological morphology.

Voir: Ecological Studies and Reviews IV. Bibliotheca Biotheoretica, Leiden E. J. BRILL, Vol. 4, p. 25-111, 1948.

Séance du 30 mars 1946 à Leyde

1. H. N. KLUYVER (Wageningen). The regulation of numbers in a population of wild birds.

Speaker's paper is based on a study of a series of data about the *Parus* population in artificial nesting-cavities of an estate over a period of thirty-two years.

Of each year not only the number of pairs is known, but also the date of the first egg of each brood, the number of eggs and the number of young ones that have flown out. The whole study comprises accurate data of more than 2500 coveys.

The study was completed by providing during eight years all the brooding birds and the young ones with coloured bands.

It was proved that vegetation-substrate and age are very important factors with many biological phenomena, such as the area of the territory, the number of eggs, the number of broods per year etc. Very remarkable is the fact that the loss or the gain of brooding birds from one breeding-season to the other does not correlate with the number of young ones flown out.

As the young titmice establish their territories as early as September, the number of young birds that can find room between the territories of the older birds is nearly every year smaller than the available number of young ones, which is as yet very big. So each year in September the whole breeding area is divided into the maximum number of territories. The more old birds there are, the less place is there for the young ones. There are, nevertheless, great fluctuations in the September-population in consequence of the fact that young birds put up with smaller territories than old ones. On a certain ground there is room for a certain number of old birds, but the real number of birds holding territory on it in autumn is higher and depends on the room left by the older birds to the younger. In one autumn in which there are many old birds, the maximum number of birds (old + young) for which there is room, lies lower than in another autumn with a small number of old birds.

So nearly every autumn there is an overpopulation of young birds, the number of which changes from year to year. Especially this overpopulation is vulnerable to predation.

The number of birds in the breeding season is of course also greatly influenced by external factors as severe winters. With the aid of modern statistical methods speaker succeeded in analysing this complex.

2. D. J. KUENEN. (Wilhelminadorp). On the ecological significance of two predators of *Metatetranychus ulmi* KOCH (*Acari*, *Tetranychidae*).

Voir: Tijdschrift voor Entomologie Vol. 88, p. 303-312, 1947.

Séance du 18 mai 1946 à Utrecht

1. J. DE WILDE (Amsterdam). Contribution to the physiology of the heart of insects, with special reference to the alary muscles.

Voir: Arch. Néerl. de Physiol. de l'homme et des animaux T. 28, p. 530-542, 1947.

2. W. G. WALTER (Amsterdam). The tensile strength of striated muscle, investigated on the gastrocnemius muscle of the frog.

Voir: Arch. Néerl. de Physiol. de l'homme et des animaux. T. 28, p. 655-669, 1947.

3. P. H. DAMSTÉ (Utrecht). Experimental modifications of the sexual cycle of the Greenfinch.

Voir: Journal of Exper. Biol. V. 24, No. 1 and 2, 20-35, 1947.

Séance du 21 septembre 1946 à Amsterdam

1. D. J. DE JONG (Utrecht). l'Influence de l'intensité de l'excitation sur la restauration du tonus du pied d'*Helix pomatia* L.

Voir: Proc. Kon. Nederl. Akad. v. Wetensch. V. 48, p. 381-393, 1945.

2. CHR. P. RAVEN and J. C. A. MIGHORST (Utrecht). Depolarization of the egg of *Limnaea stagnalis* L. by the influence of calcium.

Voir: Proc. Kon. Akad. v. Wetensch. V. 49, p. 1003-1010, 1946.

Séance du 2 novembre 1946 à Utrecht

1. L. TINBERGEN (Leyde). l'Epervier comme ennemi ravissant des oiseaux chanteurs.

Voir: Ardea, V. 34, p. 1-213, 1946.

2. Mlle J. H. BIJTEL (Groningen). The structure and the mechanism of movement of the gillfilaments in Teleostei.

Voir: Arch. Néerl. de Zoologie T. 8, livr. 3, p. 243-263, 1949.

Séance du 14 décembre 1946 à Leyde

1. D. DRESDEN (Utrecht). Some informations on the mode and site of action of D.D.T. and Gammexane.

The discovery of DDT (1939) started a new era in the control of insect pests. Its great toxicity and stability and its harmlessness to plants and mammals have not been found before in other synthetic insecticides. In connection with the importance of DDT, its mode of action in the insect is now being studied by a large number of investigators in the U.S. and in England. In Utrecht we ¹⁾ started our investigations on this problem in the beginning of 1946. Owing to the scarcity of co-operators and to the late date of the beginning of our work, our results cannot yet be published. In spite of these difficulties we are able to mention that our investigations are running quite parallel to those of our foreign colleagues. It is shown that the harmlessness to mammals, as mentioned before, is the consequence of the solubility of DDT in lipoids only. The insect cuticle, containing chitine and lipoids, behaves as a solvent and probably even acts as an adsorbing medium. The skin of Vertebrates, being a water-medium, prevents the DDT to enter the body. As soon as these differences are eliminated, for instance by injecting DDT into the body, the compound appears to be equally toxic to both Vertebrates and Insects.

Recently it was found in the U.S. that DDT acts as a stimulating agent to the proprioceptors in the cockroach (*Periplaneta americana*). It seems worth while to study this point in further detail.

We know very little about the physiological action of gammexane, the γ -isomer of hexachlorocyclohexane. It is probable that this new insecticide acts in the inositol-system, as its chemical structure shows much similarity to these compounds.

2. A. L. J. SUNIER (Amsterdam). La signification et l'agencement de jardins zoologiques.

Séance du 25 janvier 1947 à Amsterdam

1. P. KORRINGA (Bergen op Zoom). Lunar periodicity in the breeding of marine animals.

¹⁾ Univ. Lab. Comp. Phys., Director of these investigations: Dr. B. J. KRIJGSMAN.

The establishment of a system of predicting the spatfall of oysters in the Oosterschelde, the Dutch centre of oyster culture, led to the collection and checking of daily quantitative plankton samples in twelve consecutive years. Elaboration of the data thus produced that the production of oyster larvae in the Basin of the Oosterschelde appeared to show a marked periodicity in the course of the breeding season. Maxima in the production of oyster larvae can be expected to occur about 10 days after full and new moon. One of the maxima preponderates, and that appeared to be situated between June 26 and July 10 in the years under consideration (1935-1946). It is highly probable that it is the sequence of spring and neap tides which calls forth rhythmical reproduction in the oyster, and presumably differences in water pressure are the agent in this.

With the aid of the acquired knowledge on rhythmical reproduction in *Ostrea edulis*, it appeared to be possible to give an explanation for many cases of so far badly understood periodicity in the reproduction of marine animals.

Lunar periodicity demonstrating maxima once in a month only, occur in waters with little or no tidal amplitude. The case of *Platyeria dumerilii*, studied by RANZI, could be used to demonstrate that this type of lunar periodicity should be ascribed to influence of moonlight on sexual maturation, in these nocturnal animals highly sensitive to light. Other cases, included the famous Palolo-worm, could be classed in this section.

Proper analysis includes distinction between annual rhythms (length of the breeding season), monthly rhythms (lunar periodicity) and possible daily rhythms. In extreme cases the three rhythms in combination can see to a most complete concentration of reproductive activities in the species concerned, so that an entire population may spawn simultaneously during one single hour a year, thus greatly enhancing fertilization chances.

Voir: Ecological Monographs, V. 17, p. 347-381, 1947.

Séance du 3 mai 1947 à Amsterdam

1. G. O. E. LIGNAC (Leyde). About arenicochrome and its possible significance as a mesocatalyst.

The lugworm *Arenicola marina* contains in its layer of columnar epithelial cells, and also in the loose subepithelial connective tissue, a green pigment, which I have named arenicochrome. It consists of more or less globular granules, the diameter of which

varies from 0.25 to 1.30 μ . The subepithelial connective tissue contains a second pigment, differing from the arenicochrome by its yellowish to darkbrown colour. Its granules are coarser and more angular and they mostly occur in groups. This latter pigment is also found between the outer and inner muscles, especially around the bloodvessels. Morphologically and histo-chemically it can be considered as a melanin. There is a third pigment, the erythrocrurin, occurring in a dissolved state in the bloodplasm. Details are shown on a coloured plate, made by J. F. WALBURGH SCHMIDT and published in Proc. Royal Netherlands Academy of Sciences, Vol. 48, p. 406, 1945.

The green arenicochrome is changed into brownblack by oxidation. This is well shown by treating frozen sections at room temperature with 3% H_2O_2 ; the granules then quite change their colour within 24 hours. This darkbrown pigment, just as the melanin itself, is decolorized by 3% H_2O_2 and in the dark reduces a 1% AgNO_3 solution. This means that it behaves like a true melanin and that we may consider arenicochrome a premelanin which in the living lugworm occurs in a durable state. The reactions in question have been carried out on frozen sections of fixed and fresh material. Already FAUVEL in 1899 considered the brown pigment (the melanin) to be a chemical modification of the arenicochrome (which he named lipochrome jaune), which in an acid medium is indeed autoxidisable to melanin.

One may ask what biological significance should be ascribed to this premelanin. Observations of MAZZA & STOLFI on the worm *Halla parthenopaea* COSTA make it probable, especially in the light of researches on melanin formation by RAPER that a red pigment occurring in this worm in a durable state and which could be identified as a quinone, can be considered a pre-melanin too. The arenicochrome can probably, just as the hallachrome, be considered a quinoid pigment and as such both may be active hydrogentransmitters (mesocatalysts), which may play an important rôle in respiration.

Further details are to be found in my paper in Proc. Royal Netherlands Academy of Sciences, Vol. 48, p. 406–410, 1945. See further W. L. C. VEER in: Recueil des travaux chimiques des Pays-Bas, Tome 58, 9/10, 1939, and the same author in: Melanins, their chemistry and their significance, Chemisch Weekblad, Vol. 37. nr. 16, 1940. The chemical investigations are continued in my laboratory by P. VAN DUYN.

2. L. M. VAN NIEUWENHOVEN S. J. (Utrecht). Investigation into the structure and function of the anterior byssal retractor muscle of *Mytilus edulis* L.

Voir: Dissertation – DEKKER en VAN DER VEGT, Utrecht-Nijmegen, 1947.

Séance du 8 mars 1947 à Leyde

1. N. POSTMA (Utrecht). Le problème du tonus et quelques-unes de ses difficultés. La déconcentration suspendue.

Voir: Arch. Néerl. de Zoologie T. VIII, 4^e livr. p. 374.

2. E. VAN KOERSVELD (Utrecht). The bitterling (*Rhodeus amarus* BLOCH) as a testobject for steroid substances.

Voir: Dissertation – P. DEN BOER – Utrecht, 1949.

Séance du 18 octobre 1947 à Utrecht

1. A. D. VOÛTE et Mlle. A. J. TER PELKWIJK (Oosterbeek). Sur la cause de la distribution capricieuse de *Coleophora laricella* (Microlepidoptères) dans notre pays.

Voir: Ned. Bosbouwtijschrift No. 20, p. 7-15, 1948.

2. L. H. BRETSCHNEIDER (Utrecht). Das Prinzip des Elektronenmikroskopes. Anwendung und Ergebnisse der Elektronenmikroskopie.

Voir: Zeitschr. f. Mikroskopie B. 3, p. 12-22 et p. 160-175, 1948.

Symposium du 13 décembre 1947 à Amsterdam

1. J. VERWEY (Den Helder). Introduction générale sur la migration dans le règne animal.

2. H. J. KOCH (Louvain). Problèmes physiologiques de la migration.

3. W. H. VAN DOBBEN (Wageningen). l'Influence du temps sur la migration.

4. G. J. VAN OORDT (Utrecht). Experiences de "homing" chez des oiseaux migrants.

Séance du 21 février 1948 à Amsterdam

G. P. BAERENDS (Groningen). Sur le règlement de la pêche dans la mer du Nord.

Voir: Verslagen en mededelingen afd. Visserij No. 36, 1947.

Séance du 13 mars 1948 à Leyde

1. S. DIJKGRAAF (Utrecht). Ueber den Gehörsinn bei marinen Fischen.

Durch die Untersuchungen von v. FRISCH und seiner Schule hat sich bei der Elritze, sowie bei sämtlichen Ostariophysen und bei einigen weiteren Gruppen von Süßwasserfischen mit besonderen Mechanismen am Labyrinth, ein ausgezeichnetes Hörvermögen ergeben. Die obere Hörgrenze wird erst bei mehreren tausend Hz erreicht. Die Hörschärfe der Elritze ist der des Menschen annähernd gleich; die Grenze ihres Tonunterscheidungsvermögens liegt — trotz Fehlens einer Basilarmembran — bei $1/3$ bis $1/2$ Ton. Die Tonwahrnehmung hat ihren Sitz ausschliesslich in der Pars inferior (Sacculus und Lagena) des Labyrinthes. Nur tiefe und intensive Töne bis 100–150 Hz werden z.T. auch mit dem Hauttastsinn wahrgenommen. Sowohl Sacculus als Lagena sind an der Perzeption der Töne im ganzen Tonbereich beteiligt. Der Sacculus spricht praktisch nur auf die ihm von der Schwimmblase über die Weberschen Knöchelchen zugeleiteten Schwingungen an; die Lagena wird vermutlich direkt durch das "Lagenafenster" erregt.

Im Gegensatz zu den erwähnten Fischgruppen hatten Versuche über die Hörfähigkeit normaler Nicht-Ostariophysen ein bedeutend schlechteres Ergebnis. So wurde beim Aal die Hörgrenze bei 5–600 Hz gefunden; die Hörschärfe war wesentlich geringer als bei der Elritze. Nach Totalexstirpation der Labyrinth war die Hörgrenze um einige Töne auf etwa 400 Hz herabgesetzt; es schien dabei gleichgültig, ob nur die Pars inferior, oder nur die Pars superior entfernt wurde (DIESELHORST). Eigene Versuche an *Gobius paganellus* in der Zoologischen Station zu Neapel ergaben bei diesem Fisch eine Hörgrenze von 6–700 Hz; der Töne erzeugende Sciaenide *Corvina nigra* hingegen reagierte noch lebhaft auf einen Ton von 1024 Hz. (höhere Töne wurden nicht geprüft). Ausschaltung der Pars inferior verursachte bei *Gobius* keinerlei Gleichgewichtsstörung, hingegen eine Beeinträchtigung des Hörvermögens für die Höheren Töne. Ein Ton von 100 Hz. wurde nach wie vor gleich gut wahrgenommen, auch nach zusätzlicher Ausschaltung der Pars superior so wie der Seitenorgane. — Die Sacculusotolithen sind für die Tonwahrnehmung nicht unbedingt notwendig.

Auf elektrophysiologischem Gebiet wurde kürzlich das Auf-

treten von Potentialschwankungen in der Pars inferior des Kaulbarches entdeckt (DE VRIES). Sie sind synchron mit der Frequenz des dargebotenen Tones, entsprechen also ganz dem sog. Cochlea-Effekt des Säugerlabirynthes.

2. D. A. HOOIJER (Leyde). Evolution of mammals in the Quaternary of S.E. Asia.

The student of the Pleistocene and prehistoric fauna deals with the immediate forerunners of the animals constituting the Recent fauna. No great evolutionary changes are to be expected, and at the best he is able to trace certain trends in the evolution of the dentition or skeleton rarely extending beyond the limits of a species. Special reference is made here to the evolution of the dentition of the orang-utan, *Pongo pygmaeus* (HOPPIUS). More than 3000 teeth collected by EUG. DUBOIS in prehistoric caves in Central Sumatra during the years 1888-1890. The comparison with the recent teeth of the orang-utan (now confined to Northern Sumatra, and Borneo) shows that since the time of the deposition of the teeth in the Sumatran caves, viz., in the early Holocene, the teeth of the orang-utan became in the average 16 per cent smaller. In addition, it has been possible to trace several trends in the evolution of the dentition, all stamping the prehistoric orang-utan as a more simian form than the recent, still further removed in dental structure from man in the points which differentiate the recent orang-utan from recent man. The prehistoric males had bigger canines relative to those of the females than the present day animals. The lateral incisor was less reduced in relative size, and the excess in size of the second molar over the other molars, a typically simian feature that is met with in early stages of the evolution of the hominid dentition too, was much more stressed in the prehistoric orang-utan than it is now. The Pleistocene orang-utan from Java is indistinguishable in size from the recent, while the Pleistocene form from southern China and Indo-China is decidedly larger than the subfossil from Sumatra.

3. J. W. SLUITER and G. J. VAN OORDT (Utrecht). The influence of a gonadotrophin on the seasonal changes in the testis and deferent duct of the chaffinch (*Fringilla Coelebs*).

Voir: Quart. Journ. of Micr. Sc. V. 90, p. 1, 1949.

Séance du 17 avril 1948 à Utrecht

1. H. BOSCHMA (Leyde). Specific characters in *Millepora*.
Voir: Proc. Kon. Ned. Akad. v. Wetensch. V. 51, p. 818-823, 1948.

2. L. J. TOXOPEUS (Leyde). On the organization, execution and results of the third Archbold expedition to New Guinea.

Any expedition should previously be planned in such a manner that its results be optimal and costs in accordance with the object. In this respect RICHARD ARCHBOLD's expedition to Central Dutch New Guinea has set a splendid example. Losses of lives were very few, sickness never hampered the execution of schedules, and collections were safely dispatched. These collections are very rich, not only in new forms, but especially in view of the fact that they afford a better understanding of the structure of the New Guinea faunal development.

General results of a comparative study including other faunal districts were in agreement with the latest geological conception of the origin of the island, and the latter proved greatly to help towards a solution of the many puzzles of systematics. About the Pliocene New Guinea was broken up into an archipelago, and the present fauna exhibits many reminiscences to that. In Pleistocene times the Snow Mountains rose up to link the scattered parts. The ensuing general uplift isolated a series of high valleys N. of the central chain and their faunas have further developed in separate directions causing striking deviations from their original stock. The new land, above the timber line, being a "no man's land" at the time of its birth, is still scarcely populated by butterflies in striking contrast to corresponding altitudes of the Himalayas. Lower down the phenomenon of subspecies overlapping each other's districts occurred resulting in the formation of species duplicates. In lowland districts, however, interbreeding started at the places of contact between the former island faunas; this was already stated in birds by HARTERT, ROTHSCILD, PALUDAN and STRESEMANN (1936), and the same places afford similar conclusions as to butterflies.

Séance du 22 mai 1948 à Amsterdam

1. J. LEVER (Utrecht). A mathematical method for the determination of the state of activity of the thyroid gland.

Voir: Proc. Kon. Ned. Akad. v. Wetensch. V. 51, p. 1302-1309, 1948.

2. P. D. NIEUWKOOP (Utrecht). Some further data concerning the determination of the mesonephros.

Voir: Experientia V. 4, p. 391-394, 1948.

3. TH. G. N. DRESSCHER (Amsterdam). Biological survey of the eastern shore of the IJsselmeer.

Only little is known about the water fauna along the coast of the IJsselmeer. In the years 1942 and 1943 the shore between the Zwarte Water estuary and Harderwijk was investigated. While surveying, it was tried to get an idea of the number of water organisms. Evidently the water movement caused by the wind brings about an irregular distribution of the animals among the plants. For getting the right idea about their occurrence, therefore, the survey of a few little areas only is insufficient; the sum total taken from many test areas gives the most reliable figures.

A richer flora evidently corresponded with a richer fauna. In the shallow zone (till about 50 cm) we found a difference between the population occurring among the plants (*Chara*) and that on the bottom.

Symposium ethologique et physiologique; réunions communes des Sociétés zoologiques Néerlandaise et Belge du 4 et 5 octobre 1948 à Bruxelles

1. H. J. VONK, J. C. A. MIGHORST et A. P. DE GROOT (Utrecht). Le métabolisme de *Helix pomatia* L. étudié à l'aide d'eau lourde.

2. G. VAN GREMBERGEN (Gand). Le métabolisme respiratoire chez les Plathelminthes.

3. M. J. HEUTS (Louvain). Des facteurs écologiques et la formation des races chez *Gasterosteus aculeatus* L.

4. P. BRIEN (Bruxelles). La nidification chez les Protopères.

5. D. J. KUENEN (Wilhelminadorp). l'Influence de mesures de culture sur la population des Arthropodes des pommiers.

6. G. P. BAERENDS (Groningen). l'Ethologie de Cichlides.

7. J. LECLERQ (Liège). l'Ecologie et la physiologie des populations de *Tenebrio molitor*.

Séance du 13 décembre 1948 à Amsterdam

1. E. J. SLIJPER (Utrecht). Sur la distribution, les migrations et la condition des Baleines antarctiques

Voir: Proc. Kon. Akad. v. Wetensch. V. 51, p. 1033, 1114, 1310, 1948.

2. CHR. P. RAVEN (Utrecht). The influence of an electric field on the eggs of the snail *Limnaea stagnalis* L.

Voir: Proc. Kon. Akad. v. Wetensch. V. 51, p. 1077-1090, 1948.

3. J. HOFKER (Brielle). The Toothplate-Foraminifera.

Voir: ce volume, pp. — .

